Eesti Maaülikool
Estonian University of Life Sciences

DISTRIBUTION OF AND DAMAGES BY DUTCH ELM DISEASE AGENTS IN NORTHEASTERN EUROPE

JALAKASURMA TEKITAJATE LEVIK JA KAHJUSTUSED KIRDE-EUROOPAS

LIINA JÜRISOO

A Thesis
for applying for the degree of Doctor of Philosophy
in Forestry

Väitekiri
filosoofiadoktori kraadi taotlemiseks
metsanduse erialal

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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following papers which are referred to in text by Roman numerals.

I **Jürisoo, L.**, Adamson, K., Padari, A., Drenkhan, R. 2019. Health of elms and Dutch elm disease in Estonia. European Journal of Plant Pathology, 154 (3), 823-841. DOI: 10.1007/s10658-019-01707-0

II **Jürisoo, L.**, Selikhovkin, A.V., Padari, A., Shevchenko, S.V., Shcherbakova, L.N., Popovichev, B.G., Drenkhan, R. 2021. The extensive damages of elms by Dutch elm disease agents and their hybrids in north-western Russia. Urban Forestry and Urban Greening, 63, 127214. DOI: 10.1016/j.ufug.2021.127214

III **Jürisoo, L.**, Süda, I., Agan, A., Drenkhan, R. 2021. Vectors of Dutch elm disease in northern Europe. Insects, 12, 393. DOI: 10.3390/insects12050393

IV **Jürisoo, L.**, Padari, A. Drenkhan, R. 2021. Jalakasurma levikust ja ohtlikkusest Eestis (Spread and riskiness of Dutch elm disease in Estonia). Forestry Studies/Metsanduslikud Uurimused. 74. DOI: 10.2478/fsmu-2021-0006 [In Estonian].

The contributions of the authors to the papers were as follows:

Paper	Original idea and study design	Data collection	Data processing	Manuscript preparation
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IV	**LJ**, RD	**LJ**	**LJ**, AP	**LJ**, AP, RD

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ABBREVIATIONS

col1	the colony type gene
cu	ceratoulmin gene
EPPO	European and Mediterranean Plant Protection Organization
*Hph*I	enzyme for restriction endonuclease digest *cu* gene
DED	Dutch elm disease
ITS	the internal transcribed spacer
OTU	operational taxonomic unit
PCR	polymerase chain reaction
RFLP	restriction fragment length polymorphism
SSPP	species-specific polymerase chain reaction (PCR) priming
TFC	Tartu Fungal Collection in Estonian University of Life Sciences

1. INTRODUCTION

The elm (*Ulmus* spp.) – a genus of ecologically and culturally valuable forest and amenity tree species – has been under attack, mostly on the Northern Hemisphere (Brasier and Buck 2001) for more than a century (Santini and Faccoli 2013; Smith and Hulcr 2015; Wingfield et al. 2016; Martín et al. 2019) due to Dutch elm disease (DED).

This is the most devastating disease for elms all over the world (Brasier 1991; Brasier and Webber 2019). Elms have experienced two pandemics – the first at the beginning of the 20th century when 10-40% of elms were killed (Peace 1960; Gibbs 1978; Brasier 1996a; Brasier 2000a;) and the second since the second half of the 20th century (Brasier and Buck 2001) when also billions of elms were killed (Phillips and Burdekin 1992; Herald 2019). By the beginning of the 21st century (Brasier and Buck 2001; Kirisits 2013) approximately 80-90% (28 million) of mature elms had died in the UK, as well as hundreds of millions in North America (Brasier 2001; Brasier and Buck 2001). The annual cost of removing dead and severely diseased elms in the United States alone has reached $100 million (Campbell and Schlarbaum 1994) and keeping the disease under control has cost the same amount (Pimentel et al. 2005).

Without doubt *Ophiostoma ulmi* (Buisman) Nannf. was the causal agent of DED during the first pandemic. During the second pandemic, another species, recently described as *O. novo-ulmi* Brasier was the causal agent of DED in Western Europe and North America. It is now known to have two different subspecies – *O. novo-ulmi* subsp. *novo-ulmi* and *O. novo-ulmi* subsp. *americana* (Brasier et al. 2004; Brasier and Kirk 2001). Also, their hybrids were discovered and introduced thereafter (Konrad et al. 2002; Brasier and Kirk 2010).

Let´s give some examples: The health status of elms in Tallinn, Northern Estonia, worsened substantially in 2013 (Jürisoo et al. 2019). In St. Petersburg, Russia it has been deteriorating already since 1995 (Markova 2000). About 30 greenspaces were recorded as afflicted by DED in 2002: however, by the year 2017 the damage caused by the disease was 50 times more serious than 15 years before (Selikhovkin et al., 2010; Shcherbakova et al. 2019). Another example: since 1985 Malmö, Sweden has lost over 40,000 elms which account for 25% of all urban trees (Suneson, 2020).

In Sweden all native elm species (*U. glabra*, *U. laevis*, *U. minor*) are on the Red List assessed as Critically Endangered (Barstow and Harvey-Brown 2017a, b; Barstow, Rivers and Harvey-Brown 2017) because of enormous decimation due to DED (Gärdenfors 2010). DED reduced the elm population by two thirds in Southern Sweden from 1970ies until 2011, (Brunet et al. 2014) and half of the left population died in the next ten years (Ruks 2020). In Norway, DED was identified for the first time in Oslo in 1963 (Gibbs 1978), several devastating attacks were recorded at different places (Myking and Skrøppa 2007; Solheim et al. 2011). *Ophiostoma ulmi* had been documented during the second half of the 20th century and *O. novo-ulmi* subsp. *americana* several decades later (Solheim et al. 2011). Both, increasing global trade at the present time and global warming have contributed to the spread of DED.

At local scale insect vectors, mainly *Scolytus* species are the essential vectors in spreading of DED (Karnosky, 1979). It is known that global warming is causing changes in the climate system, such as increasing drought or wet periods, thus supporting the spread of pathogens and pests northwards (Caulton et al. 1998; Hanso and Drenkhan 2013; Selikhovkin et al. 2020).

In Northeastern Europe limited and unsystematic information on the health condition of elms, the causal agents of DED and its vectors has been available. Related species of *Ophiostoma ulmi sensu lato* are visually indistinguishable. Research on such related species is important as they might have different pathogenicity, geographic distribution, host range, effectiveness of host resistance and the pathogens may impose very different plant health threats that require distinct disease management strategies. These considerations were the main motivation for the thesis.

DED is one of the most investigated diseases (Bernier et al. 2014); however, there is still no effective control strategy in place to stop this pathogen. The spread and damage of DED agents are geographically different and the appropriate information is crucially valuable for working out the best control strategy. It could be more effective prevention with the help of early detection based on new molecular tools or specific primers for precise pathogen detection from biological samples or catching vector beetles with the help of more effective pheromone traps or analysing potential new vector beetles. All these aspects need more investigation; particularly as the interest in research of DED has

declined and today there are only a few research groups dealing with this topic (Bernier et al. 2014).

The thesis is a synthesis of four papers. Three of them (**Papers I, II, IV**) show that DED is devastating to the elm populations in Estonia and Northwestern Russia and analyse the distribution of the causal agents and their hybrids. The fourth paper (**Paper III**) is analysing potential vector beetles of DED. All four papers are dealing with the health issues of the elm species.

2. REVIEW OF THE LITERATURE

2.1. Elm species and their health condition in Northeastern Europe

European elms, class Dicotyledoneae, order Rosales and family Ulmaceae belong to two divergent sections – section *Blepharocarpus*, represented by *Ulmus laevis* Pallas and section *Ulmus*, represented by *Ulmus glabra* Huds. and *U. minor* Mill. (Venturas et al. 2014a). *Ulmus glabra* Huds. have more northern natural range, *U. laevis* Pall. more eastern and *U. minor* Mill. more southern natural range (Caudullo and De Rigo 2016).

Ulmus glabra and *U. laevis* belong to the Holarctic elements, Euro-Siberian sub-elements with a centre of distribution in Europe (Napierała-Filipiak et al. 2016). *Ulmus* expanded to Estonia during the Pre-Boreal period, spread rather rapidly and started to decline at the end of the Atlantic period (Saarse and Veski 2001).

Today, elms are forming 0.2% of the total volume of forest trees in Estonia (Aitsam et al. 2019). *Ulmus glabra* is spread all over Estonia, *U. laevis* is much rarer, being typical and well-established in floodplain landscapes (Mackenthun 2004; Kukk and Kull 2005) and listed as a near-threatened species in Estonia ('Eesti Punane Raamat' 2008). An Estonian researcher of monumental trees has recorded elms with the biggest trunk circumference of trees all over Estonia, some of them are registered in Estonian Nature Information System as protected trees (Relve 2011).

Elms are ecologically important trees as many different organisms are associated with them (Thor et al. 2010) incl. red-listed lichens (Jüriado et al. 2009) and endangered fungi (*Rhodotus palmatus, Hymenochaete ulmicola*) (Corfixen and Parmasto 2005; Kalamees 2011).

Traditionally elms have also been multi-purpose trees (Martín et al. 2019) and valued for their timber, suitability for coppicing, landscaping and as roadside trees (Richens 1983; Heybroek 2015; Caudullo and De Rigo 2016).

Elms are one of the main amenity tree species because they tolerate city conditions e.g. polluted air, anti-slip salts, grow on different soil types (incl. compacted) (Whiteley 2004), resist winds, recover well from mechanical damage and survive in droughts and temporary floods (Townsend and Douglass 2004; Scheffer et al. 2008; Zalapa et al. 2008; Buiteveld et al. 2015). Elms are valued species in urban spaces (Kaar 2011), as well as in rural areas (incl. historical parks) all over Estonia (Abner et al. 2007, 2012) and northwestern part of Russia (Firsov and Bulgakov 2017). They are one of the most important tree species in the green areas of St. Petersburg, Nothwestern Russia (Ignatieva and Konechnaya 2004; Trubacheva et al. 2014).

It was noted that elms started to decline in northern, southwestern and central parts of Estonia at the last turn of the century (Hanso 2006; Hanso and Drenkhan 2007). The health status of elms in St. Petersburg has worsened substantially since 1995, starting from the southern part of the city, the Pushkin region (Markova 2000).

DED was estimated as the main reason for the decline of elms in Northeastern Europe. However, information on the spread of the disease and damage to elm species in different habitats is almost unknown. It is known that elms are valuable esthetical and ecological trees in green areas and forest, but in terms of DED we have limited data on different growing conditions of elms. The population structure of elms after DED attack can be described as follows: single vigorous individuals next to re-sprouting stamps and patches of juvenile trees, especially in countryside (Nielsen and Kjær 2009).

There is a wide range of elm hybrid varieties, usually crossed with Asian elms (Martín et al. 2014), some of them are generally resistant to DED (Eisele 2018) and are recommended to be planted in green areas.

2.2. Impact of global trade and climate change on Dutch elm disease

It is possible that the threat to *Ulmus* spp. has risen in Estonia and Northwestern Russia due to the transportation of infected elm seedlings, plants or timber, similarly to what has happened in Sweden, Norway, the UK and the USA (La Porta et al. 2008; Brasier and Kirk 2010; Solheim et al. 2011; Menkis et al. 2016b).

Around the world, hosts and their possible pathogens have been moved out of their natural range and the species that have never met before are forced to co-exist now (Stukenbrock 2016). Generally, endemic species cause little or no disease on their plant hosts in their natural habitat due to long-term coevolution (King 2019), e.g. ash dieback agent *Hymenoscyphus fraxineus* in East Asia (Drenkhan et al. 2017b). New pathogens are threat to local hosts as populations of non-native species are increasing (Wingfield et al. 2015) and new pathogen species can even replace the previous (i.e. native) ones like it has happened with DED agents (Brasier and Buck 2001). On one hand, the new pathogen has more powerful effect on elms as it is more aggressive (Braschler and Hill 2007; Hemery et al. 2010); on the other hand, previously geographically isolated pathogens start to hybridize with the related species, being a major force in the evolution (Brasier 2000b, 2001). New organisms may have a different or even wider host range than the parental species (Ghelardini et al. 2016) and therefore, may have major deleterious impact on trees and forests (Ramsfield et al. 2016). Pathogen populations that have increased genetic variation usually have greater potential for evolutionary response to environmental change (King 2019).

Nowadays, due to climate change there are relatively warmer winters and springs, increased mean annual temperatures (Vose et al. 2005; Bentz and Jönsson 2015; Selikhovkin et al. 2020) that enable elms to grow also in new, more northern locations (Drobyshev 2001; Kullman 2003). Climate change, also unusual fluctuation of temperatures and heavy rains (Roloff et al. 2009) have an impact on trees making them more susceptible to pests and diseases (Hanso and Drenkhan 2013; Bentz and Jönsson 2015; Ramsfield et al. 2016) in forest ecosystems, as well as in urban areas (Sturrock 2012). These meteorological extremities have already caused different pathological effects in Estonian forests (Hanso and Drenkhan 2009, 2012, 2013; Adamson et al. 2015; Drenkhan et al. 2016; Lutter et al. 2019). In addition, climate influences the outbreaks of beetles, their aggressiveness, population dynamics and the capacity for migration (Bentz and Jönsson 2015) that has become more frequent (Biedermann et al. 2019). It is important to know that in Northern Europe elms grow in the northern limit of their natural range (Laasimer 1965; Ignatieva et al. 2011) resulting in an increase in their sensitivity to climate change and in their susceptibility to pathogens (Hanso and Drenkhan 2007, 2013).

2.3. Dutch elm disease and its agents

DED is a lethal vascular wilt disease, the first symptoms of which are yellowing and browning of leaves, a cross-section of an elm twig showing brown spots or streaks in the recent wood rings (Clinton and McCormick 1936; Stipes and Campana 1981). The first signs of DED can often only be seen on a few branches from the middle to the upper crown of the tree; however, it gradually affects the entire crown and trunk (Kirisits 2013).

It is a tracheomycosis-type disease caused by ascomycete fungi *Ophiostoma ulmi sensu lato* (Brasier 2001) that reproduces by budding, similar to yeast-like fungi and the spores spread rapidly in xylem with sap flow (Webber and Brasier 1984). In response to the fungal infection tyloses and resins are accumulated in xylem vessels that eventually block water transportation to the crown causing the tree to wilt and finally death (Sherif et al. 2014).

DED agent is known to kill trees rapidly, even during one or two seasons (Phillips and Burdekin 1992, Schmidt 2006). Mortality by DED varies according to the host (*Ulmus*) species and depends on the susceptibility and genetic variety (Martín et al. 2018), the stand density and possible rootgrafts (Santini and Faccoli 2013), as well as on the seasonality of the infection and stress factors like drought (Kirisits 2013); also, mortality is influenced by the pathogen´s spore concentration inside the tree (Flower et al. 2017).

The first known agent of DED *Ophiostoma ulmi* (Buisman) Melin & Nannf. (previous synonyms *Graphium ulmi* M.B. Schwartz, *Ceratostomella ulmi* Buisman, *Ceratocystis ulmi* (Buisman) C. Moreau, *Pesotum ulmi* (M.B. Schwartz) J.L. Crane and Schokn) (Lepik 1940a; Brasier and Buck 2001), the geographical origin of which is unclear (Masuya et al., 2010); was registered in Western Europe in 1918 (Brasier 1979). It has probably originated from East Asia (Masuya et al., 2010) and was first described in Holland in 1921 (Clinton and McCormick 1936). *Ophiostoma ulmi* spread to different parts of Europe (Schmidt 2006). In Estonia it was first found in 1939 (Lepik 1940a, 1940b), the disease had spread almost everywhere on the mainland of Estonia (Lepik 1940b; Kaar, 2011). DED was first recorded in the western and southwest regions of the USSR in 1936 (Moschenikova and Vjaznikova 2016), the agent

was probably *O. ulmi* (Brasier and Buck 2001). According to the global database of the European and Mediterranean Plant Protection Organization (EPPO) *O. ulmi* has been reported in Estonia since 1979 like in other Eastern European countries ('EPPO Global Database' 2019). The northernmost findings of *O. ulmi* are known in Norway (Solheim et al. 2011), in Sweden (Menkis et al. 2016b; 'EPPO Global Database' 2019) and Finland (Hintikka 1974). There is no evidence that DED is still present in Finland (Hantula 2021). DED was introduced in North-America and Central Asia with infected elm timber in 1920-1930's (Peace 1960; Brasier 1990).

First, *Ophiostoma ulmi* killed elms in most of the European countries (Peace 1960), but then the pathogen spread declined, apparently due to fungal viruses (Mitchell and Brasier 1994). Thereafter, step by step, *O. ulmi* was replaced by a more aggressive new pathogen *Ophiostoma novo-ulmi* (Brasier and Buck 2001) causing the second pandemic.

The second pandemic of DED, actually the current one, had begun already in the 1940s at two different locations: the Moldova–Ukraine region in Eastern Europe and the Southern Great Lakes area in North America (Brasier 1990, 1996b). In Estonia the spread and some outbreaks of "new DED" had been observed for the first time in the last decades of the last century. At that time the disease was considered insignificant. Increasing number of records suggest that a new epidemic of DED started since the second decade of the new century.

This new epidemic of DED which fast became pandemic is caused by *O. novo-ulmi*, incl. by its two subspecies, subsp. *americana* and subsp. *novo-ulmi* (Brasier et al. 2004; Brasier and Buck 2001; Martín et al. 2010). Subspecies *novo-ulmi* migrated westward across Europe reaching the Netherlands by mid-1970s; and eastward to Southwest Asia (Brasier and Buck 2001). In Estonia *O. novo-ulmi* was detected for the first time in 2006 (Hanso and Drenkhan 2007); however, not at the precise level of species and subspecies. *Ophiostoma novo-ulmi* became widespread in the second half of the last century in Southern Russia (Gibbs 1978) and later *O. novo-ulmi* subsp. *novo-ulmi* was also detected there (Brasier and Kirk 2001).

Subspecies *americana* spread across North America reaching both the East and West coasts by the 1970s and 1980s, respectively (Brasier and Buck

2001). In 1960s elm logs infected with subsp. *americana* were transported from Canada to Great Britain (Brasier and Gibbs 1973) and from Great Britain it probably spread to many other European countries.

The geographical ranges of the two *O. novo-ulmi* subspecies are overlapped in several parts of Europe (Brasier and Buck 2001) which also has induced their hybridisation (Konrad et al. 2002; Santini et al. 2005b; Martín et al. 2010), because the gene flow between them lacks strong barriers (Brasier and Buck 2001).

Reports on hybrid fungi were quite rare until the 1990s (Brasier 1995; Brasier and Buck 2001). In the eastern part of Europe, hybrids between the two subspecies of the pathogen had already been detected in Hungary (Brasier et al. 2004), Poland (Brasier and Kirk 2010), the Czech Republic (Dvořák et al. 2007), Lithuania (Motiejūnaitė et al. 2016) and Latvia (Matisone et al. 2020).

DED is developing because of hybridisation between the two subspecies of *O. novo-ulmi* and it is of significant ecological and epidemiological importance (Konrad et al. 2002). Molecular analysis is the most reliable way of detection of pathogens even if it is time-consuming and costly (Stenlid et al. 2011). Laboratory tests on the growth rate of pathogens lead to give a better understanding of the spread and aggressiveness of DED and presumably help to improve the ability to predict the invasiveness affecting forests and urban landscapes (Prospero and Cleary 2017). It is known that hybrid pathogens are more aggressive to hosts (Brasier 2008, 2012).

There is an urgent need for developing more effective molecular tools for the identification of DED agent hybrids (Konrad et al. 2002) with the help of high-throughput or hopefully portable molecular detection (Luchi et al. 2020) at an early stage of the disease development to protect the plants in the stage of prevention.

2.4. Spread of Dutch elm disease

Bark beetles (Coleoptera: Curculionidae, Scolytinae) are essential vectors in spreading of DED pathogens; however, if they are not in association with fungal pathogens (Wingfield et al. 2016), they are minor pests for broadleaved trees. Scolytid species are distributed worldwide (Heliövaara

and Peltonen 1999), the number of the species is increasing from north to south (Nikulina et al. 2015) depending on suitable tree species (Heliövaara and Peltonen 1999).

Elm bark beetles known as vectors of DED agents in Northern Europe include *S. laevis* Chaupis, *S. multistriatus* Marsham with its variety *triornatus* Eichhoff, *S. pygmaeus* Fabricius, *S. scolytus* Fabricius, *S. triarmatus* Eggers (Anderbrant and Schlyter 1987a; Menkis et al. 2016a; Webber 1990, 2004). In Europe the prevailing vectors are *Scolytus multistriatus* (Lindelöw 2012; Santini and Faccoli 2013; Menkis et al. 2016a), *S. scolytus* (Webber 1990; Waller 2013) and *S. pygmaeus* (Webber 2004; Santini and Faccoli 2013). The northernmost findings of those three beetle species are in parks of St. Petersburg city (Voolma et al. 2004; Dorofeeva 2008; Selikhovkin et al. 2014; Shcherbakova and Mandelshtam 2014).

It has been argued that Northern Europe is protected from DED because bark beetles do not occur there (Caulton et al. 1998; La Porta et al. 2008; Santini and Faccoli 2013; Martín et al. 2019); however, warmer climate has probably extended the northern range of *Scolytus* spp., as recorded in Northwest Russia (Selikhovkin et al. 2020).

The outbreak of elm bark beetles in Northwestern Russia started from the southern suburb of St. Petersburg, Pushkin in 1995 (Shcherbakova 2008; Selikhovkin et al. 2010). Elm bark beetles have not been found in Finland (Voolma et al. 2004; Hannunen and Marinova-Todorova, 2016), but several of them inhabit Sweden and Norway (Lekander et al. 1977; Anderbrant and Schlyter 1987b). In Estonia some of them were discovered already during the first half of the last century (Voolma et al. 2000, 2004).

Thus, the elm bark beetles have an essential role in the vector of DED pathogens in Northern Europe as well. The life cycle of bark beetles passes mostly in the wood or secondary phloem where female beetles create a tunnel into the bark of dying or dead elm wood and lay their eggs (Kirisits 2007; Sherif et al. 2014). After hatching into larvae, the insect feeds on sapwood and inner bark and after maturing adult elm bark beetles fly to feed on twig crotches and the inner bark of healthy elm trees transferring DED pathogen spores on the surface of their body and in their gut (Webber 1990, 2004; Moser et al. 2010; Bernier et al. 2014) to xylem tissues (Sherif et al. 2017). Although elm bark beetles are

the primary vector of DED pathogen, the fungus can also spread from infected trees to healthy elms through grafted roots (Gibbs 1978), more frequently in the areas where elms are closely spaced (Sherif et al. 2014).

Taking into consideration that an average flying distance of beetles is from 400 m to 5 or 6 km (Wolfenbarger and Jones 1943; Wollerman 1979), it is evident that some other factor is contributing to the transmission of the DED over longer distances (Menkis et al. 2016b). It is regional or global trade that increases the risk of invasion of new pests (Brasier 2008; Hemery et al. 2010) and pathogens (Rytkönen et al. 2008, 2011; Dehnen-Schmutz et al. 2010; Santini et al. 2013; Müller et al. 2016; Ghelardini et al. 2017; Liebhold et al. 2017; Drenkhan et al., 2020).

However, there is some evidence that more beetles might be the vectors of DED agents than previously thought (Jankowiak et al. 2019). Knowing the biology of Scolytinae and their suitable host trees, the range of these potential vector species of DED may be somewhat wider, because *Ophiostoma novo-ulmi* was found on an unknown vector and on other host species than elms in Poland (Chang et al. 2017; Jankowiak et al. 2019). In addition to *Scolytus* sp., host species from family Ulmaceae are inhabited by at least the following species of beetles common in Northern Europe: *Xyleborus dispar*, *Xyleborinus saxesenii* and *Trypodendron signatum* (Koch 1992; Yanovskiy 1999; Nikulina et al. 2015). These are polyphagous pests that inhabit numerous deciduous tree species. *Xyleborus dispar* can also attack healthy trees (incl. elms), especially when they are close to stressed hosts (Kühnholz et al. 2001; Speranza et al. 2009). Thus, together with elm bark beetles, there may be at least seven species of beetles in Northern Europe potentially being able to spread the DED agents. There is no scientific evidence from Northern Europe to support this fact; therefore, it should be tested.

2.5. Control possibilities of Dutch elm disease

Chemical and bio-control methods have not led to significant success in the control of DED (Blumenstein 2015; Pepori et al. 2018), researchers are vigorously looking for ways to combat DED as well-functioning control options have not been found so far.

Various methods used to control DED have not given the desired results (Pecori et al. 2017). Only some products are in use, e.g. fungicide

Arbotect-20®, Alamo (Stennes 2000), biocontrol product Dutch Trig® (Postma and Goossen-van de Geijn, 2016). However, these products do not provide elms with significant long-term efficient protection from DED either.

Prophylactic protection of mature trees can be justified only in case of protecting very valuable trees, e.g. in historical parks (Voeten et al. 2009). Attempts have been made to control DED with other antagonistic fungi (e.g. *Monographella nivalis*, *Alternaria tennuissima*); however, so far no good results have been obtained (Hubbes and Jeng 1981; Sutherland et al, 1995; Blumenstein 2015). Mycoviruses are known to significantly reduce the pathogenicity of *O. novo-ulmi* (Webber 1987; Swinton and Gilligan 1999). They have been used in the USA (Brasier 2000), but the disadvantage is that it has an inhibitory effect only on certain strains of the pathogen (Ganley and Bulman 2016).

The most effective control is rapid detection of pathogens on the first infected trees in a stand and removal of the trees as it was done in Malmö, Southern Sweden (Morgenroth and Östberg 2017). Detection at an early stage, provided pathogens occur only on a few trees and prompt removal of the diseased trees will significantly slow down the spread of the pathogen. That is why a systematic monitoring and sanitation programme should be worked out (Solheim et al. 2011). Resistant elms for local conditions (Santini et al. 2010) or planting material of *U. laevis* of local origin in Northeastern Europe could be used as an alternative control measure as this elm species is less attractive to the vector insects than other European elm species (Sacchetti et al. 1990). However, information on the elm species or origins to be considered as potential future trees in Northeastern Europe is quite limited.

It is possible that all potential *O. novo-ulmi* subsp. hybrids cannot be detected by using only *cu* and *col1* genes (Tziros et al. 2017). Thus, new and reliable studies are needed to analyse the actual population structure of DED agents in Northeastern Europe, including potential new hybrids and to test new primers and molecular techniques for quicker detection of the exact pathogen from biological samples in order to achieve better control over the pathogen (see chapter 2.3).

In the light of global trade it is very important to emphasise that the existing regulations for imported plant material must be more proactive (Roy et al. 2014).

3. AIMS OF THE STUDY

The hypotheses of the study were:

1. DED caused by species *Ophiostoma ulmi* has been replaced by new and invasive *Ophiostoma novo-ulmi* in Northeastern Europe.
2. In Estonia and Northwestern Russia *Ophiostoma novo-ulmi* is represented by two newly identified subspecies and their hybrids and their pathogenicity is different in rural area, incl. forest and urban space.
3. Pathogen hybrids between two subspecies of *Ophiostoma novo-ulmi* show higher growth rates *in vitro* than pure subspecies.
4. The mortality of hybrid elms is lower than of native elm species and the health status of *Ulmus laevis* is better than of *Ulmus glabra* in the conditions of Northeastern Europe.
5. *Scolytus* species are well known vectors for DED; however the actual number of potential vector beetles of DED is higher.

The specific aims of this study were:

1. To clarify the health status of elm species at different sampling sites and habitats in Northeastern Europe (**Papers I**, **II**);
2. To estimate the distribution of DED in Northeastern Europe and to isolate and identify the causative agents of DED in Estonia and Northwestern part of Russia at the highest possible level in terms of modern technology (**Papers I, II, III**, **IV**);
3. To assess and compare the vitality of elms affected by the two subspecies of *Ophiostoma novo-ulmi* in two non-consecutive years (**Paper I**);
4. To compare the growth rates of different agents of DED *in vitro* to assess potential pathogen agressiveness (**Paper II**);
5. To assess the potential vectors of DED agent in Northeastern Europe (**Papers I, II**, **III**).

4. MATERIALS AND METHODS

4.1. Study sites and sample collection

The sites in Estonia were selected on the basis of the published information regarding the occurrence of elms (Kukk and Kull 2005; Saarse and Veski 2001) and dendrofloristic inventories of parks (Abner et al. 2017, 2012; Jürisoo 2015; Laas and Treumuth 2006; Rist 2015) (Figure 1).

The sub-sites A1 and B1 were chosen by pathogen sub-species to analyse the effect of the appropriate pathogens on hosts under natural conditions. The time between the assessments was 24 months and both sub-sites had an area of ca. 10 ha (see Figure 1).

In Estonia urban greenspace included streets, city parks and urban forests. Selected rural habitats were located close to roads (avenues) or in rural parks (historical manor parks) and forests (**Papers I**, **IV)**.

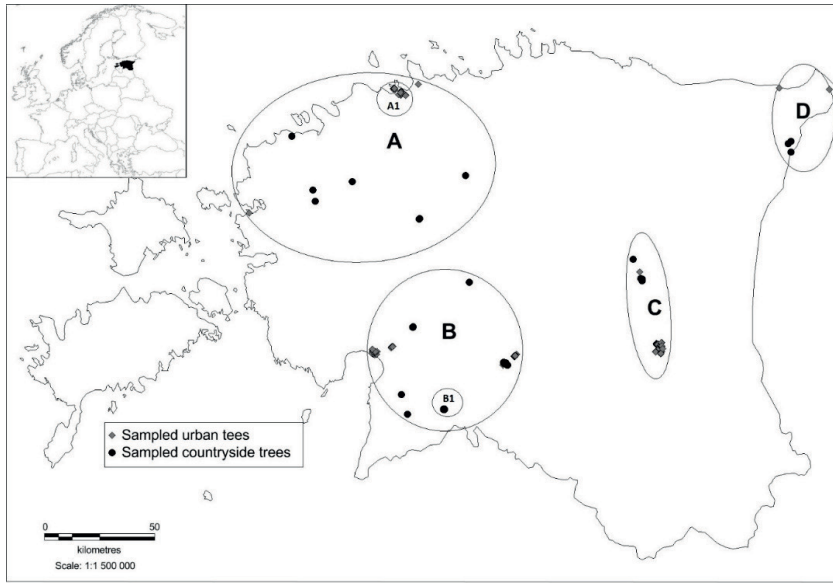

Figure 1. Locations of the four sampling sites (A, B, C, D) in Estonia. The same sample trees (N=109) were estimated in two non-consecutive years (2014 and 2016) in smaller sub-sites A1, Tallinn and B1, Tihemetsa in Estonia. Sampling site A included 15 different habitats of which 8 were in Tallinn (in A1 – 5). Sampling site B included 12 different habitats (incl. B1 – 1 habitat), sampling site C – 10 and sampling site D – 3 habitats (**Paper I**).

In Northeastern Russia the study areas (see Figure 2) in urban space included streets, city parks, alleys and greenspaces between multi-storey buildings in different regions of St. Petersburg (plots 1-9), its southern suburb Pushkin (Tsarskoje Selo, plot 1) and Viipuri (Vyborg) near the Finnish border (plot 11), rural areas along the highways from St. Petersburg to Viipuri and to Moscow (plot10) (**Paper II**).

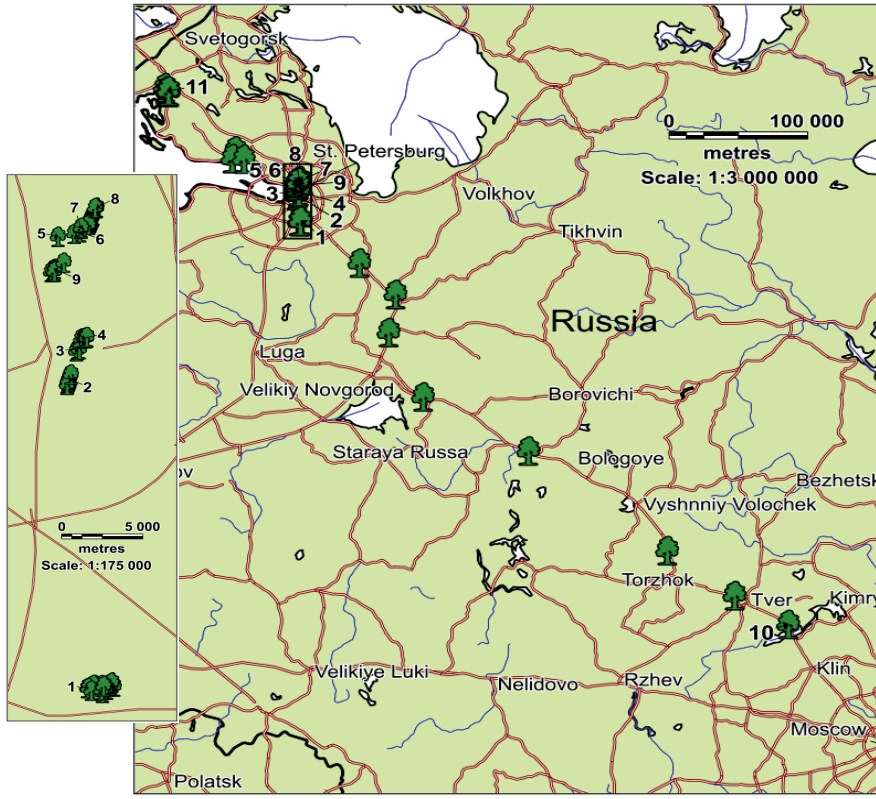

Figure 2. Sampling plots are numbered and marked with tree-shaped signs (Pitney Bowes Software, 2015) (**Paper II**).

All sampling sites of elm trees were mapped, the species identified and the crown conditions assessed. Identification of *Ulmus* species was performed according to Hillier Nurseries (1991). The precise *Ulmus* hybrids of sample trees were not identified as it is possible only by means of DNA analyses and it was not the aim of the thesis. Elm trees with foliage symptoms such as wilting, yellowing and browning of leaves were regarded as affected by DED (Solheim et al. 2011) and samples for laboratory analyses were collected from those trees (**Papers I, II, IV**).

The presence of elm bark beetles was visually determined on the trunk of every assessed tree at up to two meters height, noting the occurrence of entrance holes and larval galleries (**Papers I**, **II**).

In 2019, vector insects were caught with the help of pheromone bottle traps placed at different sites: 23 in Tallinn and 16 in other areas (39 altogether) (Figure 3) and handpicked. Traps were hung at the hight of three meters above the ground or beetles were handpicked from the trunk up to the hight of two meters (**Paper III**).

Figure 3. Bark beetles sampling sites in Estonia and St. Petersburg, Russia. Bark beetles were handpicked and collected with traps or using both methods on the same trees (**Paper III**).

4.2. Vitality assessment of elms

The health status of elms was surveyed in both countries – Estonia and Northeast Russia. Five general crown vitality classes (see Figure 4) were determined by visual assessment as follows: healthy (no visible wilting of leaves in the crown); branch loss (several branches dead and/or up to one-quarter of the crown consisting of wilting of leaves and dry branches); damaged (many dead branches and/or up to half of the crown consisting of wilting of leaves and dead branches); dying (less than a half of live branches remaining) and dead trees (no live branches remaining) (**Papers I, II**, **IV**).

Figure 4. Crown vitality classes of the surveyed *Ulmus* spp. trees (here illustrated on *U. glabra*): 1-healthy; 2-branch loss; 3-damaged; 4-dying; 5-dead (**Paper I**).

The health status of *U. glabra* was assessed twice, in summer 2014 and 2016 in sub-sites A1 and B1 in Estonia. The time between the assessments was 24 months and both sub-sites had an area of ca 10 ha (see Figure 1). The sub-species of the pathogen was determined and then the sub-sites A1 and B1 were chosen to analyse the effect of the appropriate pathogens on hosts in natural conditions.

4.3. Fungal isolation

The cultures of pathogen were isolated from the samples of the symptomatic shoots of different elms as follows: the bark of the symptomatic shoots was peeled off with a sterile scalpel and a thin layer of wood was removed up to dark brown rings in xylem. In the laboratory small pieces of the infected wood tissue were placed on sterile MEA (Malt Extract Agar, Biolife Italiana) and incubated at room temperature for 7-14 days. Subcultures were made by transferring small amounts of mycelium from colonies into new plates and incubated for ca. 14 days (**Papers I, II**, **IV)**.

4.4. Identification of beetles

All beetles caught were identified using the Olympus stereo zoom microscope SZ60 (Olympus Corporation, Japan) with 100×maximum magnification based on identification keys (Schedl 1981; Pfeffer, 1995; Voolma et al. 1997; Petrov et al. 2019). Genitals were separated when necessary and the sex of the bark beetles was determined. Total of 319 beetles were identified.

4.5. Molecular techniques

4.5.1. DNA extraction

DNA was extracted either from pure culture or separately from each individual of the beetle species using a Thermo Scientific GeneJET Genomic DNA Purification Kit (Thermo Fisher Scientific, EU). DNA was stored at -20°C until further analyses.

4.5.2. Detection of *Ophiostoma* species and other fungi

Detection of *Ophiostoma* sp. and other fungi was performed from DNA extracted from pure cultures using the fungal-specific ITS PCR primers ITS1-F (5'- CTTGGTCATTTAGAGGAAGTAA -3'; Gardes and Bruns 1993) and ITS4 (5'- CCTCCGCTTATTGATATGC -3'; White et al. 1990) and carried out as described by Drenkhan et al. (2017b).

Species-specific PCR primers mtsr1 (5'-AGTGGTGTACAGGTGAG-'3) and mtsr2 (5'-CGAGTGGTTAGTACAATCC-'3) (Gibb and Hausner 2005) were used for the detection of *O. ulmi* and *O. novo-ulmi* from mycelial DNA.

Identification of the causative agents of DED, *Ophiostoma novo-ulmi* and its subspecies was carried out from isolated cultures by species-specific PCR primers (Konrad et al. 2002). Thereafter, subspecies of *Ophiostoma novo-ulmi* were detected from mycelial DNA by the gene *col1* species-specific primed PCR (SSPP) that was performed using the primer pair F-primer (5'-GCAGTTGTTGACATGTATG-'3) and R-primer (5'-TGCTTGACGTAGATCTCG-3') described by Konrad et al. (2002).

The *cu* gene region was amplified with the primers CU1 (5'-GGGCAGCTTACCAGAGTGAAC-3') and CU2 (5'-GCGTTATGATGTAGCGGTGGC-3') (Pipe et al. 1997) and then digested by restriction enzyme *Hph* I (New England Biolabs, USA) to identify the subspecies of *Ophiostoma novo-ulmi* (see Konrad et al. 2002; Dvořák et al. 2007, and the manufacturer's instructions). The purpose of the analysis of the two genes (*col1* and *cu*) of *Ophiostoma novo-ulmi* was to detect the hybridization of the pathogen (Dvořák et al. 2007; Tziros et al. 2017).

The PCR products were visualized on 1% agarose (SeaKem® LE Agarose, Lonza) gels under UV light using the Quantum ST4-system (VilberLourmat SAS, Marne-la-allée, France). All amplifications were performed at least twice to ensure consistent banding patterns.

4.5.3. Detection of fungi from beetles using PacBio sequencing

Primers ITS4ngsUni (Tedersoo et al. 2014) and ITS1catta (Tedersoo and Anslan 2019) were used to amplify fungal DNA and the PCR products were sequenced using Sequel (Pacific Biosciences: later on PacBio) Third-Generation Sequencing in the University of Oslo in Norway. Both reverse and forward primers were equipped with 109 different MID tags with 10–12 base length (different pair per sample) that had at least 4 base differences from one another.

Conventional PCR was carried out according to Agan et al. (2020) with two replicates for each sample in 25 µl reaction volume containing 0.5 µl of forward and reverse primer and 5 µl of HOT FIREPol Blend Master Mix Ready to Load (Solis BioDyne, Tartu, Estonia). Amplification was performed as follows: 15 min at 95 °C, followed by 25 cycles of 30 s at 95 °C; 30 s at 55 °C; 1 min at 72 °C, and the final step at 72 °C for 10 min. Positive and negative controls were used throughout the analysis to exclude possible tag switches and sample contamination during the PCR process.

HTS data bioinformatics was carried out by using various programs implemented in Pipecraft v1.0, see **Paper III**.

4.5.4. Sequencing and fungal taxa conformation

PCR products from isolates of different sites and hosts were sequenced by Sanger method at the Estonian Biocentre in Tartu, using the primer ITS5 (White et al. 1990) and primers F and R for the *col1* gene (Konrad et al. 2002). The sequences were edited using the BioEdit program, Version 7.2.5 (Hall 2013) and taxa information deposited in GenBank (www.ncbi.nlm.nih.gov/genbank/) and fungal isolates in Tartu Fungal Culture Collection (TFC) (see https://natarc.ut.ee/en/seenekogud.php).

BLAST searches for the fungal taxa confirmation were performed using the GenBank database (https://www.ncbi.nlm.nih.gov/) and UNITE

v.7 database (https://unite.ut.ee/). ITS sequence similarity threshold was ≥ 99% for *Ophiostoma* spp. and ≥ 97% for other fungal species detection.

4.5.5. Fungal growth rate measurements *in vitro*

The aim of the experiment with the isolates gathered from Northwestern Russia (a total of 12 isolates, 3 different isolates of each), was to calculate the growth rate for both subspecies of *O. novo-ulmi* and their two different hybrids in eleven days. Each inoculum of an isolate was placed in the centre of a Petri dish containing 2% Malt Extract Agar (MEA) in three replicates and incubated in darkness at 21°C. Radial growth measurements of fungal colonies (Brasier and Webber 1987; Tziros et al. 2017) were recorded after four, seven and eleven days from the edge of the initial inoculum in four directions. Measurements were finished on the eleventh day when the fungal mycelia reached the wall of the Petri dish in one of the dishes (Miyashira et al. 2010). The average daily fungal growth rate was calculated for each strain of *O. novo-ulmi* subspecies and their hybrids and expressed as cm per day (**Paper II**).

4.6. Data analyses

Statistical analyses were carried out to evaluate for changes in host vitality, for probable change in tree condition, and for analyses of the impact of habitat (rural *versus* urban area) on the health of elms. The regression analyses were carried out for estimation of health condition change of elms between years 2014 and 2016 in sub-sites A1 and B1 (**Paper I**). Regression analyses was also used to generalize the course of DED positive samples throughout Estonia (**Paper IV**).

The Mann-Whitney test was carried out to evaluate the impact of different pathogen agents on the health of elms in different habitats (**Paper II**). Variance analysis (ANOVA) was used to evaluate the statistical significance of the differences in fungal growth rate of the four different DED agents *in vitro* (**Paper II**).

The percentage of *O. novo-ulmi* in vector beetles and differences between the sampling methods, sampling areas, beetle species and genders were analysed using ANOVA with Tukey HSD (**Paper III**).

5. RESULTS

In total, 2,200 mature elm trees were assessed in various urban and rural areas in Estonia: 1,225 trees over the period of 2014-2016 and 915 trees in 2018-2019. In Russia 661 elms were assessed in 2016 (Table 1).

Almost half (49%) of the surveyed trees (1094) were growing in urban space, while 1,126 (51%) trees in rural areas in Estonia. In Russia 65% (427) trees located in urban space and 35% (234) in rural areas.

Table 1. Number of assessed trees by tree species in different years in Estonia and Russia

Year	2014-2016		2018-2020	Total
Country	Estonia	Russia	Estonia	
Ulmus glabra	1020	237	915	2172
Ulmus laevis	182	265	75	522
Ulmus hybrid	20	158	5	183
Assessed trees in total	1225	661	995	2881

5.1. Agents of Dutch elm disease

In total 626 samples of the pathogen were collected from which 235 pure cultures of *Ophiostoma* spp. were successfully isolated (Table 2).

Table 2. Number of collected samples and isolated cultures of *O. novo-ulmi*.

Country	Sampling year	No of collected samples	No of isolates	*Ophiostoma novo-ulmi* subsp.			
				nu	am	nuam	amnu
Estonia	2014-2016	238	76*	14	11	0	0
Russia	2016	108	51	24	5	17	5
Estonia	2018-2020	280	108	91	16	0	1
	Total	626	235	129	32	17	6

* All isolates of *O. novo-ulmi* subspecies and their hybrids were not determined.
nu – *O. novo-ulmi* subsp. *novo-ulmi*
am – *O. novo-ulmi* subsp. *americana*
nuam – hybrid between subsp. *novo-ulmi* and subsp. *americana*
amnu – hybrid between subsp. *americana* and subsp. *novo-ulmi*

Fungal isolates were analysed with several molecular primers, resulting in the identification of the major pathogen (*Ophiostoma novo-ulmi*) in Estonia and Northwestern Russia. We did not find any isolates attributable to the species *O. ulmi*.

The distribution map (Figure 5) shows the locations of infected trees from which DED was isolated into pure culture and then identified molecularly. The first detected isolation was collected in Tihemetsa, Southewestern Estonia. *O. novo-ulmi* subsp. *americana* which is of North American origin exists only in Northwestern Estonia (see the calculated area of black-dot contour on Figure 5); however, another DED pathogen, subspecies *O. novo-ulmi* subsp. *novo-ulmi* is common in the rest of Estonia.

Ophisotoma novo-ulmi subsp. *novo-ulmi* was found in all sampling plots in Russia, except on the highway from St. Petersburg to Moscow (plot 10; Figure 1). From a total of 51 isolates 24 were identified as *O. novo-ulmi* subsp. *novo-ulmi*; five isolates belonged to subsp. *americana*. All the remaining 22 isolates were identified as hybrids between the subspecies. Seventeen of these strains were hybrids between subsp. *novo-ulmi* x *americana*. Five isolates of hybrid strains were identified as subsp. *americana* x *novo-ulmi* (**Paper II**).

The presence of an aggressive hybrid of the pathogen (*Ophiostoma novo-ulmi* subsp. *americana* x *novo-ulmi*) was identified for the first time in Estonia in 2020. It was registered in the location where the distribution of the two subspecies of the pathogen overlap (see Figure 5, **Paper IV**).

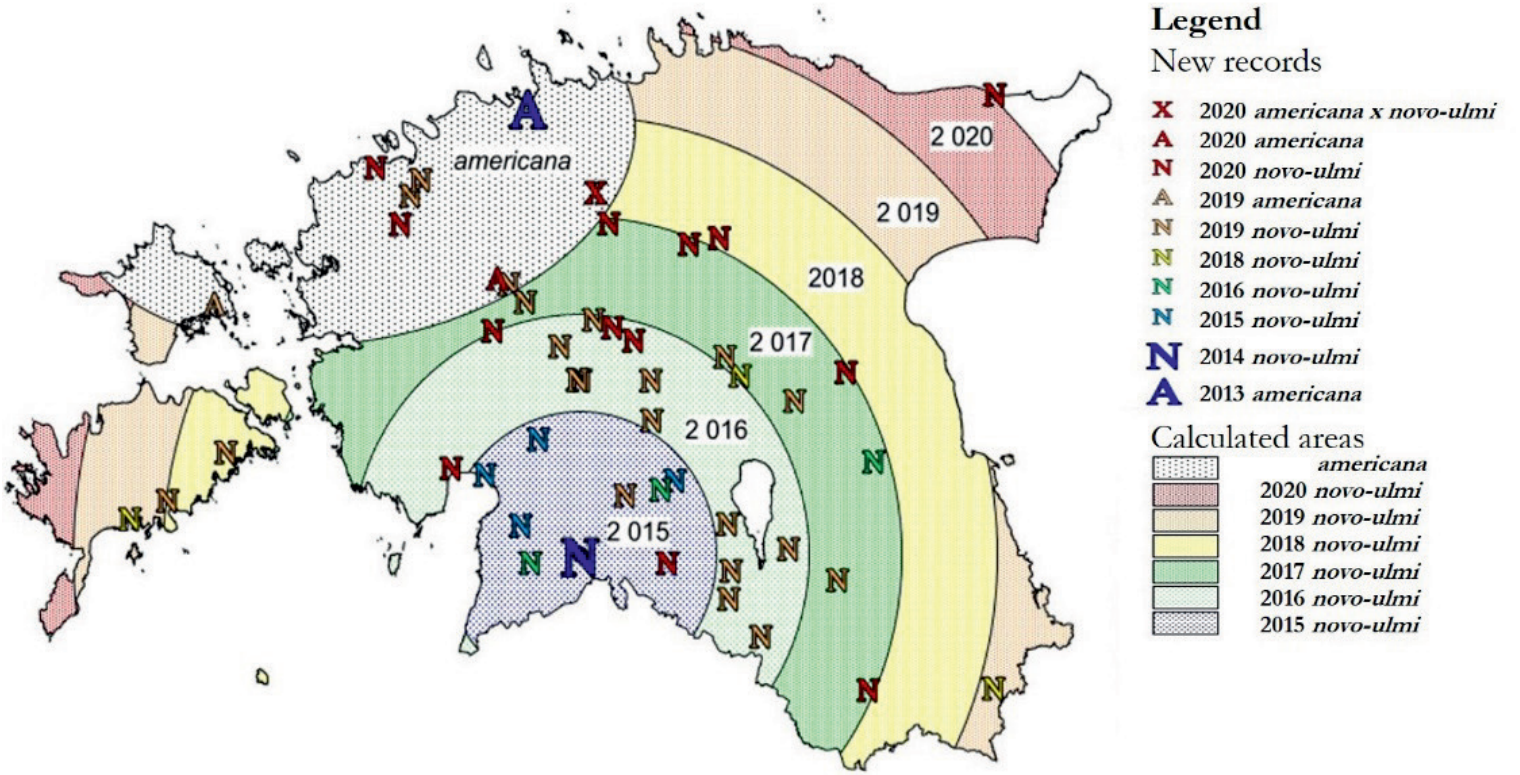

Figure 5. Location of positive samples of the DED pathogen subspecies (*Ophiostoma novo-ulmi*) ("N" – subsp. *novo-ulmi*, "A" – subsp. *americana*) and the estimated spread of the pathogen by calendar years using regression analysis. Pathogen hybrid (X) *O. novo-ulmi* subsp. *americana* x *novo-ulmi* was detected at Kose, North Estonia (**Paper IV**).

5.2. Aggressiveness of Dutch elm disease agents

Ophiostoma novo-ulmi subsp. *americana* proved to be more aggressive than *O. novo-ulmi* subsp. *novo-ulmi*. In 24 months 28% of the surveyed elms (18 trees) were found dead at sub-site A1 (Figure 1). At sub-site B1 where the damage was caused by *O. novo-ulmi* subsp. *novo-ulmi*, only 4 trees (12%) had died. Regression analyses indicated that the probability of elm trees of dying within 2 years after being infected with DED (caused by both subspecies) is ca 22%, based on the survey of 109 elm trees (**Paper I**).

The growth of the *O. novo-ulmi* subspecies *americana in vitro* was marginally but not significantly faster than of the subspecies *novo-ulmi*, 0.47 and 0.46 cm per day, respectively. The hybrids of *Ophiostoma novo-ulmi* subspecies demonstrated significantly ($p<0.0001$) higher growth rate *in vitro* compared to pure subspecies. The fastest mycelial growth was registered in *O. novo-ulmi* subsp. *americana* x *novo-ulmi,* with the mean radial growth rate of 0.63 cm per day, followed by *O. novo-ulmi* subsp. *novo-ulmi* x *americana* with 0.54 cm per day (**Paper II**). The results indicate that pathogen hybrids may have higher pathogenicity to elms compared to pure subspecies.

5.3. Health condition of different elm species in Estonia and Northwestern Russia

In Estonia, the health status of *U. laevis* is generally better than that of *U. glabra* which was also confirmed by the comparative analysis of the health status of trees in 2014-2016 (**Paper I**). The same result was obtained in Northwestern Russia (St. Petersburg) in 2016 (**Paper II**). It is also indirectly confirmed by the fact that every eleventh tree of the total number of *U. glabra* evaluated for DED was infected whereas only every 37th *U. laevis* was infected with DED.

Correlation between the DED symptoms and the vitality class of native elm species (*U. glabra* and *U. laevis*) showed that the health of elm species in Estonia (indicated by vitality class) correlated statistically significantly ($p < 0.001$) with the DED symptoms. Correlation between the symptoms of DED and the vitality class of elms in Russia showed that 89-100% of the trees without DED symptoms were in a higher vitality class (1, 2, or 3) whereas 46-72% of DED-symptomatic elm trees were in a higher

vitality class (**Paper II**). It means that visually healthy and symptomatic trees had been infected by the pathogen quite recently.

There was not any significant difference ($p > 0.05$) in the vitality of the assessed elm (*Ulmus* spp.) trees in urban and rural sites in Estonia (**Paper I**). In Russia the health situation was different, *U. glabra* was significantly healthier ($p<0.0001$) in urban areas (greenspaces or streets) versus highways in rural areas. There was some difference in the health of *U. glabra* between the habitats in urban space in Russia where street trees were significantly ($p=0.03$) better compared to the trees in greenspaces. The health of *U. laevis* was almost the same in urban space when comparing the trees on streets and in greenspaces ($p>0.05$), as well as the trees in greenspaces and highways ($p>0.05$). Also, the health of hybrid elms in urban spaces was similar ($p>0.05$) on streets and in greenspaces. The assessment of trees revealed that in Russia young hybrid elms (<25 years old) showed significantly ($p < 0.001$) higher vitality than older (>40 years old) natural species *U. laevis* and *U. glabra*. However, the mortality of the estimated hybrid elms and *U. laevis* trees was similar, 4-5% (**Paper II**).

In Estonia the regression analyses indicated that the probability of elm trees of dying within 2 years after being infected with DED (caused by both subspecies) is ca 22% based on the survey of 109 elm trees. Of all the investigated 1,225 elms *U. laevis* survived better (none of the trees were found dead) than naturally occurring *U. glabra* over the two sampling years (**Paper I**).

5.4. The beetles vectoring DED

During the first assessment period (2014-2016) all the assessed trees were examined for signs of elm bark beetles, e.g., for entrance holes, larval galleries etc. In Estonia those galleries belonged to *Scolytus multistriatus*, *S. scolytus* or *S. triarmatus* (**Paper I**).

In Russia only ten trees of *U. glabra* and 11 of *U. laevis* demonstrated colonisation attempts or entrance holes of bark beetles, the species of which were not established. However, twenty-one hybrid elms had entrance holes of *S. pygmaeus*. Larval galleries were not found on most alive trees; however, galleries of *S. scolytus* and *S. multistriatus* were found on ten dead trees. Six of those trees were colonized by both beetle species whereas four trees were colonized only by *S. multistriatus*.

81% of the beetles were handpicked and 9% trapped (Table 3) (**Paper III**). 261 of the total of 319 caught specimens were potential vector beetle individuals for DED.

Table 3. Beetle species, potentially acting as vectors for DED, caught with traps and/or handpicked from symptomatic trees.

Species of beetles	Country	Traps		Trees		Total
		Gender		Gender		
		male	female	male	female	
Scolytus multistriatus	Estonia	3	3	-	-	6
	Russia	-	-	5	-	5
Scolytus triarmatus	Estonia	4	2	66	114	186
Scolytus laevis	Estonia	-	-	21	18	39
Scolytus scolytus	Russia	-	-	2	2	4
Scolytus pygmaeus	Russia	-	-	-	1	1
Xyleborinus saxesenii	Estonia	-	10	-	-	10
Xyleborus dispar	Estonia	-	10	-	-	10
	Total	32		229		261

These beetles were selected from 109 sequenced beetles to represent different beetle species, gender, locations and different collecting methods. *Ophiostoma novo-ulmi* was found on 76 specimens, on six out of seven beetle species. Only *S. pygmaeus* carried no *O. novo-ulmi*. In total, *O. novo-ulmi* was the most prevalent fungal species on beetles according to PacBio sequencing (**Paper III**).

The highest average percentage of *O. novo-ulmi* per sample was found on the beetle species *S. scolytus,* followed by *X. saxesenii* and *S. triarmatus* with 26.6%, 20.5% and 18.2%, respectively (Figure 6). Differences in percentage of *O. novo-ulmi* among beetle species were not statistically significant, possibly due to the large variation in sample size among species. The difference between genders was also not statistically significant. The new vectoring insects of pathogen caught by traps were identified as females only. Comparing gender of the beetles, more female beetles of *S. scolytus* were carrying the pathogen than males. However, more male beetles of *S. triarmatus* compared to females had the pathogen. Other beetle species had more or less equal percentage of pathogen on individuals of different genders. Comparison of the beetles directly handpicked from

trees and collected with traps showed no significant (P>0.05) difference in the percentage of *O. novo-ulmi* (**Paper III**).

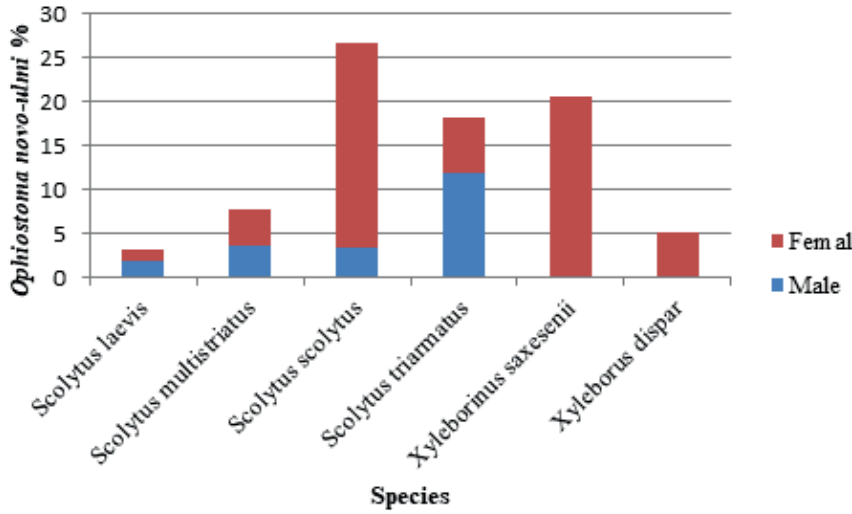

Figure 6. Percentage of *O. novo-ulmi* across different beetle species and gender (N=109). No *O. novo-ulmi* was found on *S. pygmaeus*.

6. DISCUSSION

In 2013 and DED was registered as the cause for the deterioration of the health of elms in Tallinn, Northern Estonia. Also, cases of dying elms were reported in other parts of Estonia and Saint Petersburg, Russia. My research consists of analyses of the health status of different elm species in different habitats, finding out the possible vectors spreading the disease agents and most importantly, discover the main possible cause of death of elms.

6.1. DED in Northern Europe

In Finland, DED caused by *O. ulmi* was reported in the middle of the last century (1952-1968) (Hintikka 1974), today DED agent is no longer present in Finland (Hannunen and Marinova-Todorova 2016; 'EPPO Global Database' 2019; Hantula 2021). However, in the course of this study I have detected DED in Viipuri (Vyborg), Russia, close to the Finnish border (**Paper II**).

In Estonia the areas where DED was observed in the first half of the last century (Lepik 1940b) were mostly the same as today with some minor differences (see Figure 7). In the last century, DED was not detected on the islands of Western Estonia and in Southeastern Estonia – Vastseliina. However, in towns such as Narva, Mustvee, Võru and Rapla where DED was found during the first epidemic, DED has not been detected over the past years. In Heimtali, Southern Estonia, on the left bank of Raudna primeval valley only a few *U. glabra* trees and some *U. laevis* have survived on almost two hectares. DED occurred in Oru Park (Ida-Viru County, Northeastern Estonia) at the beginning of the last century, but DED was not detected at that site in 2016. However, in 2020 infected elms were found there again. There may be various possible causes; for example, DED may not have been noticed by specialists in this park (e.g. having no clear visual symptoms), the planting material infected with DED was brought from somewhere nearby or vector insects reached the area enabling the spreading of the disease to local older trees.

DED was first registered in 1936 in western regions of Russia (Moschenikova and Vjaznikova 2016). In recent years the death of elms has been catastrophic in Northwestern Russia affecting the whole

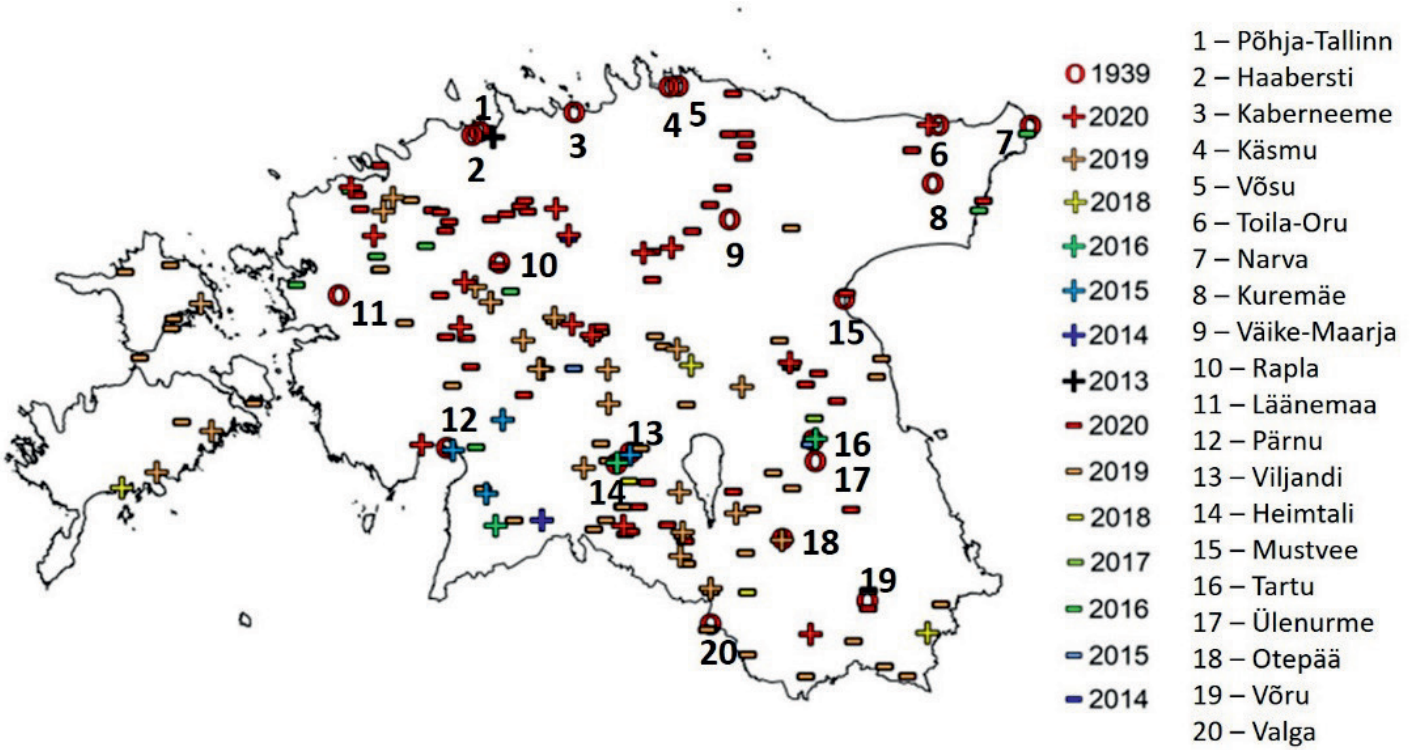

Figure 7. Records of DED in 1939 and 2013-2020 in Estonia, „O" – 1939 positive records, „+" – positive records in 2013-2020, „-" – negative records in 2013-2020 (base map Haldus- ja asustusjaotus, 2020). (**Paper IV**)

territory of St. Petersburg and the nearest suburbs (Selikhovkin et al. 2010; Shcherbakova and Mandelshtam 2014). According to the data of the Committee for City Improvement and Roads of St. Petersburg the damaged area of DED increased by 3.5 folds during the period of 2009 to 2015.

6.2. Identification of the causal agent of DED

The pure cultures of the pathogen were isolated from the shoot samples of 235 different elm specimens showing typical symptoms of DED infection. Identification of the causative agents of DED, i.e. *Ophiostoma novo-ulmi* and its subspecies was carried out on the basis of isolated cultures using species-specific PCR primers (e.g. Gibb and Hausner 2005, Konrad et al. 2002). The primers were found to be effective only in case of pure cultures and were not species-specific when testing directly from symptomatic host tissues. Therefore, species-specific PCR primers are needed for quick and reliable detection of *Ophiostoma* species and subspecies from symptomatic host tissues. These primers should be useful for fast checking of imported and exported planting material and for the general molecular monitoring of DED agents.

Detection of the hybrids of the pathogen is even more difficult and costly. The isolates of both subspecies were examined to determine the hybrids in the ceratoulmin (*cu*) and in the colony type gene (*col1*). Only these two genes were used for the detection of pathogen hybrids; however, the pathogen hybrids can also be detected by other genes.

One possibility that has recently been successfully used in metabarcoding analysis of microorganisms on plants, incl. various tree species is to use high throughput sequencing to detect pathogens from biological samples (Agan et al. 2020; Loit et al. 2019; Tedersoo et al. 2019). Third generation sequencing e.g. PacBio sequencing platform worked well in case of identifying *O. novo-ulmi* from different bark beetle species (**Paper III**) but it does not show the pathogen subspecies and the hybrids of fungi.

6.3. DED agents in Estonia and in Northwestern Russia

In the course of the studies over the period of 2014-2016 two subspecies of the invasive pathogen – *Ophiostoma novo-ulmi* subsp. *novo-*

ulmi and *O. novo-ulmi* subsp. *americana* were identified for the first time in Estonia (**Paper I**) and Northwestern Russia (**Paper II**) using molecular methods. During these studies the occurrence of *O. ulmi,* the previous agent of DED, was detected neither in Estonia nor in Russia (**Paper I, II**), the situation being similar to what has been observed elsewhere in Europe where the more aggressive *O. novo-ulmi* has displaced the earlier naturally occurring species *O. ulmi* (Brasier 2001; Brasier and Kirk 2001). Unfortunately, there is no data on the interim period and therefore; it is not possible to decide exactly when the transition from one agent to another has taken place.

During the first period of the studies (2014-2016) both subspecies were found in Estonia quite far from each other – at a distance of over 100 km. At this time *O. novo-ulmi* subsp. *americana* was found only in Tallinn, Northern Estonia (**Paper I**).

Both subspecies of *Ophiostoma novo-ulmi* were found at several sites in Northwestern Russia (**Paper II**). In case two subspecies occur in the same region, there is always a possibility of hybridization; thus, hybrids were idendified in Russia (**Paper II**). However, no pathogen hybrids were detected during 2014-2016 in Estonia (**Paper I**); only recently (in 2020) a pathogen hybrid was also found in Northern Estonia (**Paper IV**).

In Estonia the pathogen hybrid *americana* x *novo-ulmi* occurred in the overlapping distribution areas of the two different subspecies (**Paper IV**). It should be noted that the distribution map (Figure 7) does not show previous (performed before 2013) findings of the pathogen (e.g. Hanso and Drenkhan 2007; Drenkhan et al. 2017a) as the pure cultures from earlier times have not been preserved and the exact subspecies was unknown. Therefore, the results show the current situation of the pathogen spread. It is also possible that the pathogen may have spread from several infected sites. The issue of the true origin and distribution centres of the DED pathogen in Estonia and elsewhere is open for further genetic studies on the pathogen populations.

Why is the detection of pathogens or pathogen subspecies and hybrids important? For example, from 2014 to 2016 in Northern Estonia, Tallinn (sub-site A1) elms died much quicker than in sub-site B1, located in the Southwest of Estonia; ca. 25% of the assessed trees in Tallinn

were dead in 24 months. Statistically significant ($p < 0.00001$) difference between sub-sites A1 and B1 concerning the health of the elms has apparently developed due to the occurrence of different subspecies of the pathogen. The samples from sub-site A1 showed the presence of non-hybrid *O. novo-ulmi* subsp. *americana* which is recognized as more aggressive to elms (Brasier and Kirk 2001) than *O. novo-ulmi* subsp. *novo-ulmi*. Thus, the elms died significantly faster in Northern Estonia at the site where subsp. *americana* was present (**Paper I**).

The hybrids of these pathogens may indicate higher aggressiveness taking into consideration their faster growth rate *in vitro* (Gibbs and Brasier 1973; Gibbs et al. 1975; Brasier and Afsharpour 1979; Brasier and Webber 1987). For example, *O. novo-ulmi* subsp. *americana* x *novo-ulmi* showed significantly ($P<0.05$) fast mycelial growth with a mean radial growth rate of 0.63 cm per day followed by *O. novo-ulmi* subsp. *novo-ulmi* x *americana* with 0.54 cm per day. Subspecies *novo-ulmi* and *americana* grew the slowest, 0.46 and 0.47 cm per day, respectively. The growth in pure culture of the subspecies *americana* was a bit faster but the difference was not significant ($P>0.05$) between the subspecies (**Paper II**). But the subspecies *americana* is estimated to be slightly more pathogenic than *novo-ulmi* (Brasier and Buck 2001) supported by the analyses carried out in nature (**Paper I**). Thus, fast and realiable detection of pathogen subspecies and hybrids is one quite effective control measures to protect elm populations.

Higher agressiveness of the hybrids of the pathogens can evidently be proved by the inoculation tests (Gibbs et al. 1975; Brasier and Afsharpour 1979; Santini et al. 2005) but this was not the aim of the current work (**Paper II**). Special studies on artificial inoculation are needed to assess the differences in susceptibility of elm species or hybrid cultivars (McPherson et al. 2009; Griffin et al. 2017), the aggressiveness of the DED pathogens (Sherif et al. 2017) or the overall reaction of trees to the pathogen (Martín et al. 2019). Also, it is important to assess the responses of elm species of different origin to DED over the period of at least 5-10 years (Solla et al. 2005).

6.4. DED affects host species differently

Although DED is one of the most investigated diseases from many perspectives, there are still various unclarities concerning the plant-

pathogen interactions (Bernier et al. 2014). *Ulmus* species have different susceptibility to DED (Guries and Smalley 2000; Townsend 2000; Martín et al. 2014; Venturas et al. 2014b) that may depend on the anatomy and physiology of trees (Solla and Gil 2002; Martín et al. 2009, 2013), attractivity to feeding beetles (Martín-Benito et al., 2005; Martín et al., 2021), including host plant volatiles (Büchel et al. 2014, 2016). In some areas of Estonia, at heavily diseased natural elm population sites some mature elm trees seemed less susceptible to DED pathogens than others.

At the assessed sampling sites in Estonia *Ulmus glabra* was significantly more affected by DED pathogens than *U. laevis* (Figure 4) (**Paper I**); similar results have been found in Poland (Łakomy et al. 2016), Belgium (Vander Mijnsbrugge et al. 2005) and Germany (Mackenthun 2004). It can be explained by the fact that *U. glabra* has much higher hydraulic conductivity than other European elms that plays an important role in the spread of budding cells of the DED pathogens and the transport of toxins because it influences the sap flow (Solla and Gil, 2002b; Venturas et al., 2013). However, artificial inoculation tests of *U. laevis* with DED pathogen showed that some clones of this elm species are also susceptible to DED pathogens (Pinon et al. 2005; Solla et al. 2005).

The assessment of elms in nature over two non-consecutive years, 2014 and 2016, demonstrated a disastrous decline in elm tree vitality at particular sampling sites in Estonia. The trees were affected by DED, about 22% of the infected trees were dead in two years and the assessment in 2020 showed the continuity of the same tendency. The history of severe attacks of DED in other countries in Europe and North America demonstrates a similar trend (Phillips and Burdekin 1992; Schmidt 2006).

Of local native elm species *U. glabra* is more susceptible to the disease than *U. laevis* which is significantly healthier, the symptoms of DED have been found only in a few specimens (**Papers I, II**). Therefore, it is recommended to use *U. laevis* rather than *U. glabra* in landscaping. In Belgium, foresters and land managers are also increasingly interested in planting *U. laevis* (Vander Mijnsbrugge et al. 2005). More resistant hybrid elm varieties may be promising for the green areas in Northern Europe; however, tests have to be carried out before starting using these varieties more widely.

Some elm cultivars usually remain uninfected by DED pathogens, as was shown in a previous research in Estonia (Aaspõllu 1999); however, a mature *U. glabra* 'Camperdownii' died due to DED in Tihemetsa park, Southwestern Estonia (sub-site B1, Figure 5). Comparisons of different elm cultivars show a large variety of DED-resistance (Santini et al. 2005; Solla et al. 2005; Buiteveld et al. 2015). Environmental conditions including climate change events weaken the hosts, and consequently, the susceptibility of the elm cultivars to DED may increase under high disease pressure (Buiteveld et al. 2015). In St. Petersburg some hybrid elm cultivars suffered from DED as was detected in one alley where 150 trees had been planted. About 20 of those elms had died and some of the trees had typical symptoms of the DED as well as bark beetles and their holes. It is an evident indication that all hybrid elm varieties are not resistant to DED and the susceptibility of the hosts may vary between geographical locations (**Paper II**). It means that geographical provenance trials are strongly recommended to test introduced plants.

Elms of foreign provenance may be sensitive to new climatic conditions (Bowring et al. 2009). In Estonia, i.e. in Northern Baltics, dieback of shoots was noticed in case of lately planted hybrid elms (Resista) during the first years after planting. The reason may be that most of these elm hybrids have *U. pumila* as one of the parental species (Brunet et al. 2013) (e.g. *Ulmus davidiana* var. *japonica* × *U. pumila* 'New Horizon') that are sensitive to late frosts in early springs (**Paper I**). Today, six years later, those trees are not damaged. Suitability of other potential hybrid elms for Northern European climate is quite unknown and progeny trials should be set up to test them. The first attempts of planting hybrid elms in Northeastern Europe have given some hope that elms will not disappear from landscaping (Lorberg 2014).

6.5. Vectors of DED pathogen

When referring to the beetles that spread DED, we mean the species that can inhabit living elms, their bark and/or wood. Until now, the main culprits in the spread of DED agents in Europe have been *Scolytus* spp. (Lindelöw 2012; Santini and Faccoli 2013; Menkis et al. 2016a), vectoring the pathogen into visually healthy trees during their adult stage of feeding and breeding.

Elm bark beetles are widespread in greenspaces in the cities of Russia, including St. Petersburg (Mandelshtam and Popovichev 2000; Shcherbakova 2008). In Estonia the bark beetles that vector DED pathogen were reported already in 1930s and also later but mostly as individual cases (Voolma et al. 2004).

During the first assessment period (2014-2016) in Estonia it was proved that the vector beetles exist at the assessed sites; however, information about their abundance was not available. Larval galleries and entrance holes indicated that some elm beetle species do exist, but the galleries of *S. scolytus* and *S. triarmatus* are so similar that without collecting beetles it is not possible to determine on the precise species (Süda 2006). Thus, beetles were collected with pheromone traps and were handpicked from trees.

In this park, where DED was first detected in my research, one of the main vectors *S. multistriatus* was caught with the trap. It can be a indication that disease became to that region from southern areas e.g. Latvia as this species was known there (Telnov, 2004).

The number of beetle species and specimens caught with pheromone-baited bottle traps used in this study indicated quite low efficiency. That kind of traps are not suitable for catching bark beetles with the aim of exercising a control measure. The pheromones probably do not work well in the conditions of Northeastern Europe (**Paper III**). However, the trap worked well for the scientific purposes. In Estonia elm bark beetles (*S. laevis*, *S. multistriatus*, *S. triarmatus*) were registered in new locations. The spread of numerous southern beetle species, such as *S. multistriatus* in the north over the past two decades is clearly noticeable (Süda 2011) and can be a result of the climate change. In Estonia *Scolytus triarmatus* has become abundant during a very short time, considering that it occurs neither in Latvia, Lithuania nor in Finland (**Paper III**).

Using PacBio sequencing the DED pathogen has been found in six commonly captured beetle species: *S. scolytus, S. laevis, S. multisriatus, S. triarmatus, X. saxesenii*, *X. dispar* (see Figure 6). Among those, *X. saxesenii* and *X. dispar* were found as new vectors for DED in Europe. In this study *Ophiostoma novo-ulmi* was not detected on *S. pygmaeus*. The difference between the caught male and female beetles was not statistically significant (**Paper III**).

However, the caught new vector beetles (*X. saxesenii* and *X. dispar*) were only females. It could be explained by the fact that male *X. saxesenii* cannot fly at all (Sandoval et al. 2016) and only female *X. dispar* fly into traps filled with alcohol (Speranza et al. 2009). In this study alcohol was used to kill the beetles fast in trap container to minimize cross contamination risk of beetles in the same trap.

Two new vectors for DED turned to be an important finding in this work considering the importance of insect vectors in the spread of certain dangerous disease.

7. CONCLUSIONS

A more comprehensive survey on DED in Estonia started in 2014 and in Russia in 2016. In the period of 2014-2020 almost 3,000 trees from genus *Ulmus* were assessed. Over 600 samples were collected of which 235 pure cultures of *Ophiostoma novo-ulmi* were isolated and analysed with different molecular primers.

The study has provided new information on DED, it's agents, vectors and the health situation of elms in the Northeastern Europe where the invasive pathogen *O. novo-ulmi* with its two subspecies (subsp. *novo-ulmi* and subsp. *americana*) and their hybrids were identified for the first time in Estonia and Northwestern Russia.

The analyses showed that the mean probability of native elm trees of dying within 24 months after being infected with DED is ca 22%. *Ophiostoma novo-ulmi* subsp. *americana* demonstrated higher aggressiveness as its victims died more than twice faster than the elms infected by *O. novo-ulmi* subsp. *novo-ulmi*. The growth of *O. novo-ulmi* subspecies *americana in vitro* was marginally quicker than of the subspecies *novo-ulmi*. The hybrids of *O. novo-ulmi* subspecies demonstrated even faster growth rate *in vitro* than pure subspecies. This fact may indicate that hybrid pathogens are more aggressive to elms and suggests a need for precise pathogen detection.

The analyses showed that *O. novo-ulmi* subsp. *novo-ulmi* is widely spread in Estonia; however, *O. novo-ulmi* subsp. *americana* was detected only in Northwestern Estonia. Both subspecies and their hybrids are spread all over St. Petersburg´s area in Russia. The results show that the situation regarding the health of elms in Estonia and Northwestern Russia is highly alarming.

In Estonia, the health status of *U. laevis* is generally better than that of *U. glabra*, which has also been confirmed by the comparative analysis of the health status of trees in 2014 and 2016. The same result has been obtained in Northwestern Russia in 2016. Also, it has been confirmed that every eleventh evaluated *U. glabra* tree was infected with a DED agent whereas only every 37th tree of *U. laevis* was infected with the pathogen.

The results of this work regarding the known elm bark beetles (*Scolytus laevis*, *S. multistriatus*, *S. triarmatus* and *S. scolytus*) indicate that there are two new beetle species that can potentially spread DED: *Xyleborus dispar* and *Xyleborinus saxesenii*.

The first hypothesis of this study has been proved: the previous causal agent of DED *Ophiostoma ulmi* has totally been replaced by new and invasive *Ophiostoma novo-ulmi* in Estonia and Northwestern Russia (**Papers I, II, III, IV**).

The second hypothesis has been proved: also in Northeastern Europe *Ophiostoma novo-ulmi* is represented by two subspecies and their hybrids (**Papers I**, **II**, **IV**). The pathogenicity has been confirmed at two different sites with both pathogen subspecies in Estonia showing that subsp. *americana* is more aggressive to elms (**Paper I**). The vitality of the assessed elm (*Ulmus* spp.) trees was same in urban and rural sites in Estonia (**Paper I**). In Russia the health situation was different, *U. glabra* was significantly healthier in urban sites (greenspaces or streets) versus highways in rural sites, but health of *U. laevis* was almost the same in all sites (**Paper II**).

The third hypothesis has also been proved: the experiment on the growth rate of the pathogens *in vitro* also suggested that the hybrids between subspecies should be even more aggressive compared to pure pathogen subspecies (**Paper II**).

The fourth hypothesis has been proved on this part: of the two native *Ulmus* species *U. laevis* is healthier than *U. glabra* also in Northeastern Europe (**Papers I**, **II**, **IV**). Mortality of hybrid elms is lower compared to *U. glabra* but the same compared to *U. laevis* (**Papers I**, **II**).

The fifth hypothesis has been proved: two new vector beetles for DED have been discovered, these are *Xyleborinus saxesenii* and *Xyleborus dispar* (**Paper III**).

Some applied suggestions based on the thesis are as follows:

1. Systematic inventory of elm trees at national level helps rapid identification of DED. Elms should be assessed at least once a year, preferably in summer, in forest and urban space.
2. The spread of DED can be decreased with the help of a sanitation programme involving fast removal of affected trees.
3. Mass trapping of vector beetles is also one possibility to slow down the spread of DED; however, the method is effective only in case there are isolated bark beetle populations (El-Sayed et al. 2006); the trapping methods need further work in Northern European conditions. Removing of DED infected and bark beetle infested elms from green areas would be a more effective control measure.
4. Targeted regulation of free trade through legislation and a control system prevents introduction of new pathogens through adventitious and unknown plant material, it also contributes to the reduction of the risk of DED; in addition, the plants must meet the quality requirements for the nursery plants (EVS 939-2 2020).
5. Current work results suggest that native *U. laevis* should be planted in urban space and rural areas instead of *U. glabra*.
6. It is recommended to test new elm hybrids in progeny trials in local conditions before using them routinely in urban green areas in Northern European conditions.
7. New and reliable species-specific DNA primers or alternatively, high throughput sequencing is needed for quick pathogen detection from biological samples to ensure faster control of the spread of the disease.
8. Precise detection of more aggressive pathogen subspecies and hybrids is crucially important for better protection of elm populations, the infected trees should be removed and destroyed as fast as possible.
9. Educating the public would also be needed to understand the need for early removal of the diseased trees, even if they still seem healthy for a nonprofessional in order to restrict the spread of this dangerous pathogen of elms in green areas and forests.

REFERENCES

Abner, O., Konsa, S., Lootus, K., Sinijärv, U. 2007. Eesti pargid 1 (Estonian Parks 1) . Keskkonnaministeerium, Muinsuskaitseamet, Varrak, Tallinn. 423 pp. [In Estonian]

Abner, O., Konsa, S., Lootus, K., Sinijärv, U. 2012. Eesti pargid 2 (Estonian Parks 2) . Keskkonnaministeerium, Muinsuskaitseamet, Varrak, Tallinn. 647 pp. [In Estonian]

Adamson, K., Drenkhan, R., Hanso, M. 2015. Invasive brown spot needle blight caused by *Lecanosticta acicola* in Estonia. Scand. J. For. Res. 30, 587–593. https://doi.org/10.1080/02827581.2015.1041550

Agan, A., Drenkhan, R., Adamson, K., Tedersoo, L., Solheim, H., Børja, I., Matsiakh, I., Timmermann, V., Nagy, N.E., Hietala, A.M. 2020. The relationship between fungal diversity and invasibility of a foliar niche—the case of ash dieback. J. Fungi 6, 1–27. https://doi.org/10.3390/jof6030150

Aitsam, V., Sims, A., Tolm, T., Nikopensius, M., Karu, H., Raudsaar, M., Valgepea, M., Timmusk, T., Pärt, E. 2019. Statistical forest: 20 years of statistical forest inventory in Estonia (Statistiline mets: 20 aastat statistilist metsainventeerimist Eestis) . Keskkonnaagentuur. 151 pp. [In Estonian]

Anderbrant, O., Schlyter, F. 1987a. Differences in morphology and sexual size dimorphism between the Dutch elm disease vectors *Scolytus laevis* and *Scolytus scolytus* (Col., Scolytidae). J. Appl. Entomol. 103, 378–386. https://doi.org/10.1111/j.1439-0418.1987.tb00999.x

Anderbrant, O., Schlyter, F. 1987b. Ecology of the Dutch Elm Disease Vectors *Scolytus laevis* and *S. scolytus* (Coleoptera: Scolytidae) in Southern Sweden. J. Appl. Ecol. 24, 539. https://doi.org/10.2307/2403891

Anderbrant, O., Yuvaraj, J.K., Martin, J.A., Gil, L., Witzell, J. 2017. Feeding by *Scolytus* bark beetles to test for differently susceptible elm varieties. J. Appl. Entomol. 141, 417–420. https://doi.org/10.1111/jen.12354

Bentz, B.J., Jönsson, A.M. 2015. Modeling Bark Beetle Responses to Climate Change, in: Bark Beetles. Academic Press, pp. 533–553. https://doi.org/10.1016/B978-0-12-417156-5.00013-7

Bernier, L., Aoun, M., Bouvet, G.F., Comeau, A., Dufour, J., Naruzawa, E.S., Nigg, M., Plourde, K. V. 2014. Genomics of the dutch elm disease pathosystem: Are we there yet? IForest. https://doi.org/10.3832/ifor1211-008

Biedermann, P.H.W., Müller, J., Grégoire, J.C., Gruppe, A., Hagge, J., Hammerbacher, A., Hofstetter, R.W., Kandasamy, D., Kolarik, M., Kostovcik, M., Krokene, P., Sallé, A., Six, D.L., Turrini, T., Vanderpool, D., Wingfield, M.J., Bässler, C. 2019. Bark Beetle Population Dynamics in the Anthropocene: Challenges and Solutions. Trends Ecol. Evol. 34(10), 914-924

Blumenstein, K. 2015. Endophytic fungi in elms: implications for the integrated management of Dutch elm disease. Southern Swedish Forest Research Centre, Swedish University of Agricultural Sciences. 84 pp.

Bowring, J., Egoz, S., Ignatieva, M. 2009. 'As good as the West': two paradoxes of globalisation and landscape architecture in St. Petersburg. Journal of Landscape Architecture 4, 6–15. https://doi.org/10.1080/18626033.2009.9723409

Braschler, B., Hill, J.K. 2007. Role of larval host plants in the climate-driven range expansion of the butterfly *Polygonia c-album*. J. Anim. Ecol. 76, 415–423. https://doi.org/10.1111/j.1365-2656.2007.01217.x

Brasier, C.M. 1979. Dual origin of recent Dutch elm disease outbreaks in Europe. Nature 281, 78–80. https://doi.org/10.1038/281078a0

Brasier, C.M. 1990. China and the origins of Dutch elm disease: an appraisal. Plant Pathol. 39, 5–16. https://doi.org/10.1111/j.1365-3059.1990.tb02470.x

Brasier, C.M. 1991. *Ophiostoma novo-ulmi* sp. nov., causative agent of current Dutch elm disease pandemics. Mycopathologia 115, 151–161. https://doi.org/10.1007/BF00462219

Brasier, C.M. 1995. Episodic selection as a force in fungal microevolution, with special reference to clonal speciation and hybrid introgression. Can. J. Bot. 73, 1213–1221. https://doi.org/10.1139/b95-381

Brasier, C.M. 1996a. New horizons in Dutch elm disease control, Report on Forest Research 1996. HMSO.

Brasier, C.M. 1996b. Low genetic diversity of the *Ophiostoma novo-ulmi* population in North America. Mycologia 88, 951–964. https://doi.org/10.1080/00275514.1996.12026736

Brasier, C.M. 2000a. Intercontinental Spread and Continuing Evolution of the Dutch Elm Disease Pathogens, in: Dunn, C.P. (Ed.), The Elms: Breeding, Conservation, and Disease Management. Springer US, pp. 61–72. https://doi.org/10.1007/978-1-4615-4507-1_4

Brasier, C.M. 2000b. The rise of the hybrid fungi. Nature 405, 134–135. https://doi.org/10.1038/35012193

Brasier, C.M. 2000c. Viruses as biological control agents of the Dutch elm disease fungus Ophiostoma novo-ulmi, in: Dunn, C.P. (Ed.), The Elms - Breeding, Conservation and Disease Management. Kluwer Academic Publishers, Boston, USA, pp. 201–212.

Brasier, C.M. 2001. Rapid Evolution of Introduced Plant Pathogens via Interspecific Hybridization is leading to rapid evolution of Dutch elm disease and other fungal plant pathogens. Bioscience 51, 123–133. https://doi.org/10.1641/0006-3568(2001)051[0123:reoipp]2.0.co;2

Brasier, C.M. 2008. The biosecurity threat to the UK and global environment from international trade in plants. Plant Pathol. 57, 792–808. https://doi.org/10.1111/j.1365-3059.2008.01886.x

Brasier, C.M. 2012. Rapid Evolution of Introduced Tree Pathogens Via Episodic Selection and Horizontal Gene Transfer, in: Sniezko, R.A., Yanchuk, A.D., Kliejunas, J.T., Palmieri, K.M., Alexander, J.M., Frankel, S.J. (Eds.), Proceedings of the 4th International Workshop on Genetics of Host-Parasite Interactions in Forestry. Pacific Sothwest Research Station, Forest Service, U.S. Department of Agriculture., Albany, CA, pp. 133–142.

Brasier, C.M., Afsharpour, F. 1979. The aggressive and non-aggressive strains of *Ceratocystis ulmi* in Iran. Eur. J. For. Pathol. 9, 113–122. https://doi.org/10.1111/j.1439-0329.1979.tb00668.x

Brasier, C.M., Buck, K.W. 2001. Rapid Evolutionary Changes in a Globally Invading Fungal Pathogen (Dutch Elm Disease). Biol. Invasions 3, 223–233. https://doi.org/10.1023/A:1015248819864

Brasier, C.M., Buck, K., Paoletti, M., Crawford, L.J., Kirk, S. 2004. Molecular analysis of evolutionary changes in populations of *Ophiostma novo-ulmi*. Invest. Agrar. Sist. Recur. 13, 93–103. https://doi.org/10.5424/817

Brasier, C.M., Gibbs, J.N. 1973. Origin of the Dutch elm disease epidemic in Britain. Nature 242, 607–609. https://doi.org/10.1038/242607a0

Brasier, C.M., Kirk, S.A. 2001. Designation of the EAN and NAN races of *Ophiostoma novo-ulmi* as subspecies. Mycol. Res. 105, 547–554. https://doi.org/10.1017/S0953756201004087

Brasier, C.M., Kirk, S.A. 2010. Rapid emergence of hybrids between the two subspecies of *Ophiostoma novo-ulmi* with a high level of pathogenic fitness. Plant Pathol. 59, 186–199. https://doi.org/10.1111/j.1365-3059.2009.02157.x

Brasier, C.M., Webber, J.F. 1987. Positive correlations between in vitro growth rate and pathogenesis in *Ophiostoma ulmi*. Plant Pathol. 36, 462–466. https://doi.org/10.1111/j.1365-3059.1987.tb02263.x

Brasier, C.M., Webber, J.F. 2019. Is there evidence for post-epidemic attenuation in the Dutch elm disease pathogen *Ophiostoma novo-ulmi*? Plant Pathol. 68, 921–929. https://doi.org/10.1111/ppa.13022

Brunet, J., Bukina, Y., Hedwall, P.O., Holmström, E., von Oheimb, G. 2014. Pathogen induced disturbance and succession in temperate forests: Evidence from a 100-year data set in southern Sweden. Basic Appl. Ecol. 15, 114–121. https://doi.org/10.1016/j.baae.2014.02.002

Brunet, J., Zalapa, J.E., Pecori, F., Santini, A. 2013. Hybridization and introgression between the exotic Siberian elm, *Ulmus pumila*, and the native Field elm, *U. minor*, in Italy. Biol. Invasions 15, 2717–2730. https://doi.org/10.1007/s10530-013-0486-z

Büchel, K., Austel, N., Mayer, M., Gershenzon, J., Fenning, T.M., Meiners, T. 2014. Smelling the tree and the forest: elm background odours affect egg parasitoid orientation to herbivore induced terpenoids. BioControl 59, 29–43. https://doi.org/10.1007/s10526-013-9544-9

Büchel, K., Fenning, T., Gershenzon, J., Hilker, M., Meiners, T. 2016. Elm defence against herbivores and pathogens: morphological, chemical and molecular regulation aspects. Phytochem. Rev. https://doi.org/10.1007/s11101-015-9442-0

Buiteveld, J., Van Der Werf, B., Hiemstra, J.A. 2015. Comparison of commercial elm cultivars and promising unreleased Dutch clones for resistance to *Ophiostoma novo-ulmi*. iForest - Biogeosciences For. 8, 158–164. https://doi.org/10.3832/ifor1209-008

Campbell, F.T., Schlarbaum, S.E. 1994. Fading forests North American trees and the threat of exotic pests. Natural Resources Defense Council, New York.

Caudullo, G., De Rigo, D. 2016. *Ulmus* - elms in Europe: distribution, habitat, usage and threats, in: San-Miguel-Ayanz, J., de Rigo, D., Caudullo, G., Durrant, H.T., Mauri, A. (Eds.), European Atlas of Forest Tree Species. Luxembourg.

Caulton, E., Aitken, W., Rashid, N. 1998. Aerobiological aspects of elm (*Ulmus* spp) in South-East Scotland in relation to elm decline from Dutch elm disease (1976–1996). Aerobiologia (Bologna). 14, 147–153. https://doi.org/10.1007/BF02694199

Chang, R., Duong, T.A., Taerum, S.J., Wingfield, M.J., Zhou, X., De Beer, Z.W. 2017. Ophiostomatoid fungi associated with conifer-infesting beetles and their phoretic mites in Yunnan, China. MycoKeys 28, 19–64. https://doi.org/10.3897/mycokeys.28.21758

Clinton, G.P., McCormick, F.A. 1936. Dutch elm disease. *Graphium ulmi*. Bulletin (Connecticut Agricultural Experiment Station), 389. New Haven.

Corfixen, P., Parmasto, E. 2005. *Hymenochaete ulmicola* sp. nov. (Hymenochaetales). Mycotaxon 91, 465–469.

Dehnen-Schmutz, K., Holdenrieder, O., Jeger, M.J., Pautasso, M. 2010. Structural change in the international horticultural industry: Some implications for plant health. Sci. Hortic. (Amsterdam). 125, 1–15. https://doi.org/10.1016/j.scienta.2010.02.017

Dorofeeva, T.B. 2008. Dutch elm disease epiphytoty in St. Petersburg (Epifitotija ofiostomoza v Sankt-Peterburge). Zashita i karantin rasteniy (Protection Quar. plants) 3, 59. [in Russian]

Drenkhan, R., Adamson, K., Drenkhan, T., Agan, A., Laas, M. 2017a. Uus probleemistik dendropatoloogias – uued ja invasiivsed patogeenid. (New problems in dendropathology – new and invasive pathogens) Forestry Studies/Metsanduslikud Uurim. 67, 50–71. [In Estonian]

Drenkhan, R., Ganley, B., Martín-García, J., Vahalík, P., Adamson, K., et al. 2020. Global geographic distribution and host range of *Fusarium circinatum*, the causal agent of pine pitch canker. Forests. https://doi.org/10.3390/F11070724

Drenkhan, R., Riit, T., Adamson, K., Hanso, M. 2016. The earliest samples of *Hymenoscyphus albidus* vs. *H. fraxineus* in Estonian mycological herbaria. Mycol. Prog. 15, 835–844. https://doi.org/10.1007/s11557-016-1209-5

Drenkhan, R., Solheim, H., Bogacheva, A., Riit, T., Adamson, K., Drenkhan, T., Maaten, T., Hietala, A.M. 2017b. *Hymenoscyphus fraxineus* is a leaf pathogen of local *Fraxinus* species in the Russian Far East. Plant Pathol. 66, 490–500. https://doi.org/10.1111/ppa.12588

Drobyshev, I.V. 2001. Effect of natural disturbances on the abundance of Norway spruce (*Picea abies* (L.) Karst.) regeneration in nemoral forests of the southern boreal zone. For. Ecol. Manage. 140, 151–161. https://doi.org/10.1016/S0378-1127(00)00324-8

Dvořák, M., Tomšovský, M., Jankovský, L., Novotný, D. 2007. Contribution to identify the causal agents of Dutch elm disease in the Czech Republic. Plant Prot. Sci. 43, 142–145.

Eesti Punane Raamat (Red Data Book) [WWW Document] 2008. Eesti Tead. Akad. Looduskaitse Komisjon. URL https://vana.elurikkus.ut.ee/prmt.php (accessed 13.03.21). [In Estonian]

Eisele 2018. Elms - lungs of the city. [WWW Document] URL http://resista-ulmen.com/wp-content/uploads/E_Resista_Image_Brochure_Web.pdf (accessed 29.07.2019)

El-Sayed, A.M., Suckling, D.M., Wearing, C.H., Byers, J.A. 2006. Potential of Mass Trapping for Long-Term Pest Management and Eradication of Invasive Species. J. Econ. Entomol. 99, 1550–1564. https://doi.org/10.1093/jee/99.5.1550

EPPO Global Database [WWW Document] 2019. EPPO. URL https://gd.eppo.int/ (accessed 6.7.19).

EVS 939-2 2020. PUITTAIMED HALJASTUSES Osa 2: Ilupuude ja -põõsaste istikute kvaliteedinõuded (WOODEN PLANTS IN LANDSCAPING Part 2: Quality requirements for ornamental trees and shrubs) . Eesti Standardimis- ja Akrediteerimiskeskus. 26 pp. [In Estonian]

Firsov, G., Bulgakov, T. 2017. The modern state of elms (*Ulmus* L., Ulmaceae) in arboretum of Peter the Great Botanic Garden under conditions of epiphytoty of the Dutch elm disease. Hortus Bot. 12, 278–312. https://doi.org/10.15393/j4.art.2017.3962

Ganley, R.J., Bulman, L.S. 2016. Dutch elm disease in New Zealand: impacts from eradication and management programmes. Plant Pathol. 65, 1–9. https://doi.org/10.1111/ppa.12527

Gärdenfors, U. 2010. Rödlistade arter i Sverige 2010 (The 2010 Red List of Swedish Species). SLU Publikationsservice, 199 pp. [In Swedish]

Gardes, M., Bruns, T.D. 1993. ITS primers with enhanced specificity for basidiomycetes - application to the identification of mycorrhizae and rusts. Mol. Ecol. 2, 113–118. https://doi.org/10.1111/j.1365-294X.1993.tb00005.x

Ghelardini, L., Luchi, N., Pecori, F., Pepori, A.L., Danti, R., Della Rocca, G., Capretti, P., Tsopelas, P., Santini, A. 2017. Ecology of invasive forest pathogens. Biol. Invasions 19, 3183–3200. https://doi.org/10.1007/s10530-017-1487-0

Ghelardini, L., Pepori, A.L., Luchi, N., Capretti, P., Santini, A. 2016. Drivers of emerging fungal diseases of forest trees. For. Ecol. Manage. https://doi.org/10.1016/j.foreco.2016.09.032

Gibb, E.A., Hausner, G. 2005. Optional mitochondrial introns and evidence for a homing-endonuclease gene in the mtDNA rnl gene in *Ophiostoma ulmi* s. lat. Mycol. Res. 109, 1112–1126. https://doi.org/10.1017/S095375620500376X

Gibbs, J.N. 1978. Intercontinental Epidemiology of Dutch Elm Disease. Annu. Rev. Phytopathol. 16, 287–307. https://doi.org/10.1146/annurev.py.16.090178.001443

Gibbs, J., Brasier, C.M. 1973. Correlation between cultural characters and pathogenicity in *Ceratocystis ulmi* from Britain, Europe and America. Nature 241, 381–383. https://doi.org/10.1038/241381a0

Gibbs, J.N., Brasier, C.M., McNabb, H.S., Heybroek, H.M. 1975. Further studies on pathogenicity in *Ceratocystis ulmi*. Eur. J. For. Pathol. 5, 161–174. https://doi.org/10.1111/j.1439-0329.1975.tb00461.x

Griffin, J.J., Jacobi, W.R., Mcpherson, E.G., Sadof, C.S., Mckenna, J.R., et al. 2017. Ten-Year Performance of the United States National Elm Trial, Arboriculture & Urban Forestry.

Guries, R.P., Smalley, E.B. 2000. Once and Future Elms: Classical and Molecular Approaches to Dutch Elm Disease Resistance, in: The Elms. Springer US, Boston, MA, pp. 231–248. https://doi.org/10.1007/978-1-4615-4507-1_14

Hannunen, S., Marinova-Todorova, M. 2016. Pest Risk Assessment for Dutch elm disease. Finnish Food Safety Authority Evira, Helsinki. 110 pp.

Hanso, M., 2006. Süütu kottseen ja ohtlik jalakahaigus. (Innocent carcinoma and dangerous disease of elms). Maaleht: Metsaleht 6. [In Estonian]

Hanso, M., Drenkhan, R. 2007. Metsa- ja linnapuud ilmastiku äärmuste vaevas (Trees in forests and towns are suffering from the extreme weather conditions). Eesti Lood. (Estonian Nature) 58, 6–13. [In Estonian]

Hanso, M., Drenkhan, R. 2009. *Diplodia pinea* is a new pathogen on Austrian pine (*Pinus nigra*) in Estonia. Plant Pathology, 58(4), p. 797. https://doi.org/10.1111/j.1365-3059.2009.02082.x

Hanso, M., Drenkhan, R. 2012. Lophodermium needle cast, insect defoliation and growth responses of young Scots pines in Estonia. For. Pathol. 42, 124–135. https://doi.org/10.1111/j.1439-0329.2011.00728.x

Hanso, M., Drenkhan, R. 2013. Simple visualization of climate change for improving the public perception in forest pathology. For. Stud. 58, 37–45. https://doi.org/10.2478/fsmu-2013-0004

Hantula, J. 2021. The story of Dutch elm disease (Hollaninjalavataudi tarina) [In Finnish]. Sorbifolia 52, 31–35.

Heliövaara, K., Peltonen, M. 1999. Bark Beetles in a Changing Environment 47, pp.48-53. Ecol. Bull. https://doi.org/10.2307/20113226

Hemery, G.E., Clark, J.R., Aldinger, E., Claessens, H., Malvolti, M.E., O'Connor, E., Raftoyannis, Y., Savill, P.S., Brus, R. 2010. Growing scattered broadleaved tree species in Europe in a changing climate: a review of risks and opportunities. Forestry 1, 65–81. https://doi.org/10.1093/forestry/cpp034

Herald 2019. Hundreds of Dutch elm disease trees to be felled. Her. Scotl. Online.

Heybroek, H. 2015. The elm, tree of milk and wine. iForest - Biogeosciences For. 8, 181–186. https://doi.org/10.3832/ifor1244-007

Hillier Nurseries 1991. The Hillier manual of trees & shrubs., 6th ed. David & Charles, Winchester.

Hintikka, V. 1974. *Ceratocystis ulmi* in Finland. Karstenia 14, 32–32. https://doi.org/10.29203/ka.1974.87

Hubbes, M., Jeng, R.S. 1981. Aggressiveness of *Ceratocystis ulmi* strains and induction of resistance in *Ulmus americana*. For. Pathol. 11, 257–264. https://doi.org/10.1111/j.1439-0329.1981.tb00094.x

Ignatieva, M., Konechnaya, G. 2004. Floristic Investigations of Historical Parks in Floristic Investigations of Historical Parks in St. Petersburg, Russia. Urban Habitats 2(1), pp. 174-216.

Ignatieva, M., Konechnaya, G., Stewart, G. 2011. St. Petersburg, in: Plants and Habitats of European Cities. Springer New York, New York, NY, pp. 407–452. https://doi.org/10.1007/978-0-387-89684-7_12

Jankowiak, R., Strzałka, B., Bilański, P., Kacprzyk, M., Wieczorek, P., Linnakoski, R. 2019. Ophiostomatoid fungi associated with hardwood-infesting bark and ambrosia beetles in Poland: Taxonomic diversity and vector specificity. Fungal Ecol. 39, 152–167. https://doi.org/10.1016/j.funeco.2019.02.001

Jürisoo, L. 2015. Heimtali pargi dendroloogiline hinnang (Dendrological assessment of Heimtali park). 25 pp. [In Estonian]

Jürisoo, L., Adamson, K., Padari, A., Drenkhan, R. 2019. Health of elms and Dutch elm disease in Estonia. Eur. J. Plant Pathol. 154, 823–841. https://doi.org/10.1007/s10658-019-01707-0

Jürisoo, L., Selikhovkin, A.V., Padari, A., Shevchenko, S.V., Shcherbakova, L.N., Popovichev, B.G., Drenkhan, R. 2021b. The extensive damages of elms by Dutch elm disease agents and their hybrids in north-western Russia. Urban Forestry and Urban Greening, 63, 127214.

Jürisoo, L., Süda, I., Agan, A., Drenkhan, R. 2021b. Vectors of Dutch elm disease in northern Europe. Insects, 12, 393. DOI: 10.3390/insects12050393

Jürisoo, L., Padari, A. Drenkhan, R. 2021c. Spread and riskiness of Dutch elm disease in Estonia. Forestry Studies/Metsanduslikud Uurimused. Accepted May 26, 2021.

Kaar, E. 2011. Jalakas ja künnapuu Eestis (Elms in Estonia). Eesti Lood. (Estonian Nature) 62. [In Estonian]

Kalamees, K. 2011. Roosa võrkheinik: seenharuldus jalakal (Wrinkled Peach: rare fungus on elms). Eesti Lood. (Estonian Nature) 62, 41–41. [In Estonian]

Karnosky, D.F. 1979. Dutch Elm Disease: A Review of the History, Environmental Implications, Control, and Research Needs. Environ. Conserv. 6, 311–322.

King, K.M. 2019. Pathogen population biology research can reduce international threats to tree health posed by invasive fungi. Outlooks Pest Manag. 30, 5–9. https://doi.org/10.1564/v30_feb_02

Kirisits, T. 2007. Fungal Associates of European Bark Beetles With Special Emphasis on the Ophiostomatoid Fungi, in: Bark and Wood Boring Insects in Living Trees in Europe, a Synthesis. Springer Netherlands, pp. 181–236. https://doi.org/10.1007/978-1-4020-2241-8_10

Kirisits, T. 2013. Dutch elm disease and other ophiostoma diseases, in: Gonthier, P., Nicolotti, G. (Eds.), Infectious Forest Diseases. CABI Publishing, pp. 256–282. https://doi.org/10.1079/9781780640402.0256

Koch, K. 1992. Die Kaefer Mitteleuropas. Ökologie, 3rd ed. Goecke & Evers, Krefeld.

Konrad, H. Kirisits, T., Riegler, M., Halmschlager, E., Stauffer, C., 2002. Genetic evidence for natural hybridization between the Dutch elm disease pathogens *Ophiostoma novo-ulmi* ssp. *novo-ulmi* and *O. novo-ulmi* ssp. *americana*. Plant Pathol. 51, 78–84.

Kühnholz, S., Borden, J.H., Uzunovic, A. 2001. Secondary ambrosia beetles in apparently healthy trees: Adaptations, potential causes and suggested research, in: Integrated Pest Management Reviews. Springer, pp. 209–219. https://doi.org/10.1023/A:1025702930580

Kukk, T., Kull, T. 2005. Eesti taimede levikuatlas (Atlas of the Estonian Flora). Eesti Maaülikooli Põllumajandus- ja Keskkonnainstituut, Tartu. [In Estonian]

Kullman, L. 2003. Changes in alpine plant cover - Effects of climate warming. Sven. Bot. Tidskr. 97, 210–221.

La Porta, N., Capretti, P., Thomsen, I.M., Kasanen, R., Hietala, A.M., Von Weissenberg, K. 2008. Forest pathogens with higher damage potential due to climate change in Europe. Can. J. Plant Pathol. 30, 177–195. https://doi.org/10.1080/07060661.2008.10540534

Laas, E., Treumuth, S. 2006. Voltveti mõisapargi ja Tihemetsa arboreetumi dendroloogiline inventuur (Dendrological inventory of Voltvet manor park and Tihemetsa arboretum). Tartu. [In Estonian]

Łakomy, P., Kwaśna, H., Kuźmiński, R., Napierała-Filipiak, A., Filipiak, M., Behnke, K., Behnke-Borowczyk, J. 2016. Investigation of *Ophiostoma* population infected elms in Poland. Dendrobiology 76, 137–144. https://doi.org/10.12657/denbio.076.013

Lekander, B., Bejer-Petersen, B., Kangas, E., Bakke, A. 1977. The distribution of bark beetles in the Nordic countries. Acta Entomol. Fenn. 32, 37.

Lepik, E. 1940a. Estonia. The Elm disease in the country. Int. Bull. Plant Prot. 14, 2.

Lepik, E. 1940b. Miks kuivavad meie jalakad? (Why are our elms drying out?). Postimees 160, 5. [In Estonian]

Liebhold, A.M., Brockerhoff, E.G., Kalisz, S., Nuñez, M.A., Wardle, D.A., Wingfield, M.J. 2017. Biological invasions in forest ecosystems. Biol. Invasions 19, 3437–3458. https://doi.org/10.1007/s10530-017-1458-5

Lindelöw, Å. 2012. Introduced or Overlooked? New Bark Beetle Species in Sweden (Coleoptera; Curculionidae). Forstschutz aktuell 55, 28–29.

Loit, K., Adamson, K., Bahram, M., Puusepp, R., Anslan, S., Kiiker, R., Drenkhan, R., Tedersoo, L. 2019. Relative performance of Oxford Nanopore MinION vs. Pacific Biosciences Sequel third-generation sequencing platforms in identification of agricultural and forest pathogens. Appl. Environ. Microbiol. 85, e01368-19. https://doi.org/10.1101/592972

Lorberg 2014. 200 Resista-Elms for " Euroroute R1 " Concept.

Luchi, N., Ioos, R., Santini, A. 2020. Fast and reliable molecular methods to detect fungal pathogens in woody plants. Appl. Microbiol. Biotechnol. https://doi.org/10.1007/s00253-020-10395-4

Lutter, R., Drenkhan, R., Tullus, A., Jürimaa, K., Tullus, T., Tullus, H. 2019. First record of *Entoleuca mammata* in hybrid aspen plantations in hemiboreal Estonia and stand–environmental factors affecting its prevalence. Eur. J. For. Res. 138, 263–274. https://doi.org/10.1007/s10342-019-01165-7

Mackenthun, G.L. 2004. The role *Ulmus laevis* in German floodplain landscapes. Invest Agrar Sist Recur 13, 55–63.

Mandelshtam, M.Y., Popovichev, B.G. 2000. Annotated list of bark beetle species (Coleoptera, Scoytidae) of the Leningrad Region. Entomol. Rev. 80, 887–903.

Markova, T. 2000. Why do elms and oaks die? (Pochemu gibnut vyazy i duby?). Tsarskoselskaya Newsp. 103 (9128). [in Russian]

Martín-Benito, D., García-Vallejo, M.C., Pajares, J.A., López, D. 2005. Triterpenes in elms in Spain. Can. J. For. Res. 35, 199–205. https://doi.org/10.1139/x04-158

Martín, J.A., Domínguez, J., Solla, A., Brasier, C.M., Webber, J.F., Santini, A., Martínez-Arias, C., Bernier, L., Gil, L. 2021. Complexities underlying the breeding and deployment of Dutch elm disease resistant elms. New For. 7, 1–36. https://doi.org/10.1007/S11056-021-09865-Y

Martín, J.A., Fuentes-Utrilla, P., Gil, L., Witzell, J. 2010. Ecological factors in Dutch elm disease complex in Europe - a review. Ecol. Bull. 53, 209–224.

Martín, J.A., Sobrino-Plata, J., Rodríguez-Calcerrada, J., Collada, C., Gil, L. 2019. Breeding and scientific advances in the fight against Dutch elm disease: Will they allow the use of elms in forest restoration? New For. 50, 183–215. https://doi.org/10.1007/s11056-018-9640-x

Martín, J.A., Solla, A., Esteban, L.G., de Palacios, P., Gil, L. 2009. Bordered pit and ray morphology involvement in elm resistance to *Ophiostoma novo-ulmi*. Can. J. For. Res. 39, 420–429. https://doi.org/10.1139/X08-183

Martín, J.A., Solla, A., Ruiz-Villar, M., Gil, L. 2013. Vessel length and conductivity of *Ulmus* branches: Ontogenetic changes and relation to resistance to Dutch elm disease. Trees - Struct. Funct. 27, 1239–1248. https://doi.org/10.1007/s00468-013-0872-2

Martín, J.A., Solla, A., Venturas, M., Collada, C., Domínguez, J., Miranda, E., Fuentes, P., Burón, M., Iglesias, S., Gil, L. 2014. Seven ulmus minor clones tolerant to Ophiostoma novo-ulmi registered as forest reproductive material in Spain. IForest 8, 172–180. https://doi.org/10.3832/ifor1224-008

Masuya, H., Brasier, C.M., Ichihara, Y., Kubono, T., Kanzaki, N. 2010. First report of the Dutch elm disease pathogens *Ophiostoma ulmi* and *O. novo-ulmi* in Japan. Plant Pathol. 59, 805–805. https://doi.org/10.1111/j.1365-3059.2009.02239.x

Matisone, I., Kenigsvalde, K., Zaļuma, A., Burņeviča, N., Šņepste, I., Matisons, R., Gaitnieks, T. 2020. First report on the Dutch elm disease pathogen *Ophiostoma novo-ulmi* from Latvia. For. Pathol. e12601. https://doi.org/10.1111/efp.12601

McPherson, G., Costello, L., Harding, J., Dreistadt, S., Flint, M.L., Mezger, S. 2009. National elm trial: Initial report from Northern California. West. Arborist Fall, 32–36.

Menkis, A., Östbrant, I.L., Davydenko, K., Bakys, R., Balalaikins, M., Vasaitis, R. 2016a. *Scolytus multistriatus* associated with Dutch elm disease on the island of Gotland: phenology and communities of vectored fungi. Mycol. Prog. 15, 1–8. https://doi.org/10.1007/s11557-016-1199-3

Menkis, A., Östbrant, I.L., Wågström, K., Vasaitis, R. 2016b. Dutch elm disease on the island of Gotland: monitoring disease vector and combat measures. Scand. J. For. Res. 31, 237–241. https://doi.org/10.1080/02827581.2015.1076888

Mitchell, A.G., Brasier, C.M. 1994. Contrasting structure of European and North American populations of Ophiostoma ulmi. Mycol. Res. 98, 576–582. https://doi.org/10.1016/S0953-7562(09)80482-6

Miyashira, C.H., Tanigushi, D.G., Gugliotta, A.M., Santos, D.Y.A.C. 2010. Comparison of radial growth rate of the mutualistic fungus of *Atta sexdens rubropilosa* forel in two culture media. Brazilian J. Microbiol. 41, 506–511. https://doi.org/10.1590/S1517-83822010000200035

Morgenroth, J., Östberg, J. 2017. Measuring and monitoring urban trees and urban forests, in: Ferrini, F., Bosch, C.C.K. van den, Fini, A. (Eds.), Routledge Handbook of Urban Forestry. Routledge, pp. 33–48.

Moschenikova, N.B., Vjaznikova, E. V. 2016. Methodological manual for study of Dutch elm disease (Metodicheskoye posobiye po izucheniyu gollandskoy bolezni vyazov) [In Russian].

Moser, J.C., Konrad, H., Blomquist, S.R., Kirisits, T. 2010. Do mites phoretic on elm bark beetles contribute to the transmission of Dutch elm disease? Naturwissenschaften 97, 219–227. https://doi.org/10.1007/s00114-009-0630-x

Motiejūnaitė, J., Kutorga, E., Kasparavičius, J., Lygis, V., Norkutė, G. 2016. New records from Lithuania of fungi alien to Europe. Mycotaxon 131, 49–60. https://doi.org/10.5248/131.49

Müller, M.M., Hamberg, L., Hantula, J. 2016. The susceptibility of European tree species to invasive Asian pathogens: a literature based analysis. Biol. Invasions 18, 2841–2851. https://doi.org/10.1007/s10530-016-1174-6

Myking, T., Skrøppa, T. 2007. Variation in phenology and height increment of northern *Ulmus glabra* populations: Implications for conservation. Scand. J. For. Res. 22, 369–374. https://doi.org/10.1080/02827580701672121

Napierała-Filipiak, A., Filipiak, M., Łakomy, P., Kuźmiński, R., Gubański, J. 2016. Changes in elm (*Ulmus*) populations of mid-western Poland during the past 35 years. Dendrobiology 76, 145–156. https://doi.org/10.12657/denbio.076.014

Nielsen, L.R., Kjær, E.D. 2009. Gene flow and mating patterns in individuals of wych elm (Ulmus glabra) in forest and open land after the influence of Dutch elm disease. Conserv. Genet. 2009 11(1), 257–268. https://doi.org/10.1007/S10592-009-0028-5

Nikulina, T., Mandelshtam, M., Petrov, A., Nazarenko, V., Yunakov, N. 2015. A survey of the weevils of Ukraine. Bark and ambrosia beetles (Coleoptera: Curculionidae: Platypodinae and Scolytinae). Zootaxa 3912, 1–61. https://doi.org/10.11646/zootaxa.3912.1.1

Peace, T.R. 1960. The status and development of Elm Disease in Britain. For. Comm. Bull. 33, 44.

Pecori, F., Ghelardini, L., Luchi, N., PEPORI, A.L., Santini, A. 2017. Lights and Shadows of a Possible Strategy to Cope with Alien and Destructive Forest Pathogens: the Example of Breeding for Resistance to Dutch Elm Disease in Italy. Balt. For. 23, 255–263.

Pepori, A.L., Bettini, P.P., Comparini, C., Sarrocco, S., Bonini, A., Frascella, A., Ghelardini, L., Scala, A., Vannacci, G., Santini, A. 2018. *Geosmithia-Ophiostoma*: a New Fungus-Fungus Association. Microb. Ecol. 75, 632–646. https://doi.org/10.1007/s00248-017-1062-3

Petrov, A. V., Mandelshtam, M.Y., Beaver, R.A. 2019. A key to species of the tribe Scolytini Latreille, 1804 (Coleoptera: Curculionidae: Scolytinae) from Russia and adjacent countries. Russ. Entomol. J. 28, 286–302. https://doi.org/10.15298/rusentj.28.3.08

Pfeffer, A. 1994. Familie: Scolytidae, in: Lohse, G.A., Lucht, W.H. (Eds.), Die Käfer Mitteleuropas, 3. Supplementband Mit Katalogteil. Goecke & Evers, Krefeld, pp. 153–180.

Pfeffer, A. 1995. Zentral- und Westpaläarktische Borken- und Kernkäfer (Coleoptera: Scolytidae, Platypodidae). Pro Entomologia, Basel.

Phillips, D.H., Burdekin, D.A. 1992. Diseases of Forest and Ornamental Trees. Palgrave Macmillan UK, London. https://doi.org/10.1007/978-1-349-06177-8

Pimentel, D., Zuniga, R., Morrison, D. 2005. Update on the environmental and economic costs associated with alien-invasive species in the United States. Ecol. Econ. 52, 273–288. https://doi.org/10.1016/j.ecolecon.2004.10.002

Pinon, J., Husson, C., Collin, E. 2005. Susceptibility of native French elm clones to Ophiostoma novo-ulmi. Ann. For. Sci. 62, 689–696. https://doi.org/10.1051/forest:2005066

Pipe, N.D., Buck, K.W., Brasier, C.M. 1997. Comparison of the cerato-ulmin (*cu*) gene sequences of the Himalayan Dutch elm disease fungus *Ophiostoma himal-ulmi* with those of *O. ulmi* and *O. novo-ulmi* suggests that the *cu* gene of *O. novo-ulmi* is unlikely to ha. Mycol. Res. 101, 415–421. https://doi.org/10.1017/S0953756296002754

Postma, J., Goossen-van de Geijn, H. 2016. Twenty-four years of Dutch Trig® application to control Dutch elm disease. BioControl 61, 305–312. https://doi.org/10.1007/s10526-016-9731-6

Prospero, S., Cleary, M. 2017. Effects of host variability on the spread of invasive forest diseases. Forests 8, 80. https://doi.org/10.3390/f8030080

Ramsfield, T.D., Bentz, B.J., Faccoli, M., Jactel, H., Brockerhoff, E.G. 2016. Forest health in a changing world: Effects of globalization and climate change on forest insect and pathogen impacts. Forestry 89, 245–252. https://doi.org/10.1093/forestry/cpw018

Relve, H. 2011. Eesti jämedaimad jalakad ja künnapuud (The thickest elms in Estonia). Est. Nat. (Eesti Loodus) 12. [In Estonian]

Richens, R.H. 1983. Elm. Cambridge University Press (CUP), Cambridge.

Rist, E. 2015. Tihemetsa pargi inventuur (Inventory of Tihemetsa park). Tihemetsa. [In Estonian]

Roloff, A., Korn, S., Gillner, S. 2009. The Climate-Species-Matrix to select tree species for urban habitats considering climate change. Urban For. Urban Green. 8, 295–308. https://doi.org/10.1016/J.UFUG.2009.08.002

Roy, B.A., Alexander, H.M., Davidson, J., Campbell, F.T., Burdon, J.J., Sniezko, R., Brasier, C.M. 2014. Increasing forest loss worldwide from invasive pests requires new trade regulations. Front. Ecol. Environ. https://doi.org/10.1890/130240

Ruks, J. 2020. Effects of ash dieback and Dutch elm disease on forest structure in Dalby Söderskog 2012-2020. SLU, Southern Swedish Forest Research Centre.

Rytkönen, A., Lilja, A., Drenkhan, R., Gaitnieks, T., Hantula, J. 2011. First record of *Chalara fraxinea* in Finland and genetic variation among isolates sampled from Åland, mainland Finland, Estonia and Latvia. For. Pathol. 41, 169–174. https://doi.org/10.1111/j.1439-0329.2010.00647.x

Rytkönen, A., Lilja, A., Petäistö, R.L., Hantula, J. 2008. Irrigation water and Phytophthora cactorum in a forest nursery. Scand. J. For. Res. 23, 404–411. https://doi.org/10.1080/02827580802419034

Saarse, L., Veski, S. 2001. Spread of broad-leaved trees in Estonia. Proc. Est. Acad. Sci. Geol. 50, 51–65.

Sacchetti, P., Tiberi, R., Mittempergher, L. 1990. Preference of *Scolytus multistriatus* (Marsham) during the gonad maturation phase between two species of elm. Redia 73, 347–354.

Sandoval, O.A.M., Carrillo, D., Hulcr, J., Lucky, A. 2016. Fruit-tree pinhole borer - Xyleborinus saxesenii Ratzeburg, 1837 (Insecta: Coleoptera: Curculionidae: Scolytinae) [WWW Document]. Entomol. Nematol. URL http://entnemdept.ufl.edu/creatures/trees/beetles/Xyleborinus_saxesenii.htm (accessed 11.16.20).

Santini, A., Faccoli, M. 2013. Dutch elm disease and elm bark beetles: a century of association. http://iforest.sisef.org/ 8, 126. https://doi.org/10.3832/IFOR1231-008

Santini, A., Fagnani, A., Ferrini, F., Ghelardini, L., Mittempergher, L. 2005. Variation among Italian and French elm clones in their response to *Ophiostoma novo-ulmi* inoculation. For. Pathol. 35, 183–193. https://doi.org/10.1111/j.1439-0329.2005.00401.x

Santini, A., Ghelardini, L., De Pace, C., Desprez-Loustau, M.L., Capretti, P., et al. 2013. Biogeographical patterns and determinants of invasion by forest pathogens in Europe. New Phytol. 197, 238–250. https://doi.org/10.1111/j.1469-8137.2012.04364.x

Santini, A., Pecori, F., Pepori, A.L., Ferrini, F., Ghelardini, L. 2010. Genotype × environment interaction and growth stability of several elm clones resistant to Dutch elm disease. For. Ecol. Manage. 260, 1017–1025. https://doi.org/10.1016/J.FORECO.2010.06.025

Schedl, K.E. 1981. Familie: Scolytidae (Borken- und Ambrosiakäfer), in: Freude, H., Harde, K.W., Lohse, G.A. (Eds.), Die Käfer Mitteleuropas. Goecke & Evers, Krefeld, pp. 34–99.

Scheffer, R.J., Voeten, J.G.W.F., Guries, R.P. 2008. Biological Control of Dutch Elm Disease. Plant Dis. 92, 192–200. https://doi.org/10.1094/PDIS-92-2-0192

Schmidt, O. 2006. Wood and Tree Fungi. Biology, Damage, Protection and Use. Springer-Verlag, Berlin, Heidelberg.

Selikhovkin, A. V., Drenkhan, R., Mandelshtam, M.Y., Musolin, D.L. 2020. Invasions of insect pests and fungal pathogens of woody plants into the northwestern part of European Russia. Vestn. Saint Petersbg. Univ. Earth Sci. 65, 263–283. https://doi.org/10.21638/spbu07.2020.203 [In Russian]

Selikhovkin, A. V., Popovichev, B.G., Davydova, I.A., Neverovskiy, V.J. 2010. Outbreak of elm bark beetles in St. Petersburg (Massovoye razmnozheniye vyazovykh zabolonnikov v Sankt-Peterburge) [in Russian]. Bull. Int. Acad. Saf. Sci. 14, 5–12.

Selikhovkin, A. V., Popovichev, B.G., Lukmazova, E.A. 2014. The bark beetle *Scolytus pygmaeus* (Fabricius, 1787) in Summer Garden of St. Petersburg (Zabolonnik-pigmey *Scolytus pygmaeus* (Fabricius, 1787) (Coleoptera: Curculionidae: Scolytidae) v Letnem sadu Sankt-Peterburga), in: Musolin, D.L., Selikhovkin, A. V. (Eds.), Proceedings of the International Conference The Kataev Memorial Readings – VIII. Pests and Diseases of Woody Plants in Russia, Saint Petersburg (Russia). Saint Petersburg State Forest Technical University, Saint Petersburg (Russia), pp. 73–73. [In Russian]

Shcherbakova, L.N. 2008. Elm bark beetles in city green areas of St. Petersburg (Vyazovyye zabolonniki v gorodskikh posadkakh Sankt-Peterburga). Izv. Sankt-Peterburgskoj Lesoteh. Akad. (Transactions

Saint Petersbg. For. Tech. Acad. 182, 306–313. [in Russian, Abstract in English]

Shcherbakova, L.N., Mandelshtam, M.Y. 2014. Elms of Saint Petersburg: after the third ring (Vyazy Sankt Peterburga: posle tret'yego zvonka), in: Musolin, D.L., Selikhovkin, A. V. (Eds.), Proceedings of the International Conference The Kataev Memorial Readings – VIII. Pests and Diseases of Woody Plants in Russia, Saint Petersburg (Russia). Saint Petersburg State Forest Technical University, Saint Petersburg (Russia). [In Russian]

Shcherbakova, L.N., Shevchenko, S. V., Moschenikova, N.B. 2019. The problem of death of elms in Saint Petersburg (Problema gibeli vyazov v Sankt-Peterburge. Proc. St. Petersbg. For. Acad. (Izvestiya Sankt-Peterburgskoy lesotekhnicheskoy Akad. 228, 352–366. [In Russian]

Sherif, S., Jones, A.M.P., Shukla, M.R., Saxena, P.K. 2014. Establishment of invasive and non-invasive reporter systems to investigate American elm -Ophiostoma novo-ulmi interactions. Fungal Genet. Biol. 71, 32–41. https://doi.org/10.1016/j.fgb.2014.08.005

Sherif, S.M., Erland, L.A., Shukla, M.R., Saxena, P.K. 2017. Bark and wood tissues of American elm exhibit distinct responses to Dutch elm disease. Sci. Rep. 7. https://doi.org/10.1038/s41598-017-07779-4

Smith, S.M., Hulcr, J. 2015. *Scolytus* and other Economically Important Bark and Ambrosia Beetles, in: Vega, F.E., Hofstetter, R.W. (Eds.), Bark Beetles Biology and Ecology of Native and Invasive Species. Academic Press, pp. 495–531. https://doi.org/https://doi.org/10.1016/B978-0-12-417156-5.00012-5

Solheim, H., Eriksen, R., Hietala, A.M. 2011. Dutch elm disease has currently a low incidence on wych elm in Norway. For. Pathol. 41, 182–188. https://doi.org/10.1111/j.1439-0329.2010.00650.x

Solla, A., Bohnens, J., Collin, E., Diamandis, S., Franke, A., Gil, L., Burón, M., Santini, A., Mittempergher, L., Pinon, J., Vanden Broeck, A. 2005. Screening European Elms for Resistance to Ophiostoma novo-ulmi. For. Sci. 51, 134–141. https://doi.org/10.1093/forestscience/51.2.134

Solla, A., Gil, L. 2002a. Influence of water stress on Dutch elm disease symptoms in Ulmus minor. Can. J. Bot. 80, 810–817. https://doi.org/10.1139/b02-067

Solla, A., Gil, L. 2002b. Xylem vessel diameter as a factor in resistance of Ulmus minor to Ophiostoma novo-ulmi. For. Pathol. 32, 123–134. https://doi.org/10.1046/J.1439-0329.2002.00274.X

Speranza, S., Bucini, D., Paparatti, B. 2009. New observation on biology of European shot-hole borer [Xyleborus dispar (F.)] on hazel in northern latium (Central Italy). Acta Hortic. 845, 539–542. https://doi.org/10.17660/ActaHortic.2009.845.84

Stenlid, J., Oliva, J., Boberg, J.B., Hopkins, A.J.M. 2011. Emerging Diseases in European Forest Ecosystems and Responses in Society. Forests 2, 486–504. https://doi.org/10.3390/f2020486

Stennes, M.A. 2000. Dutch Elm Disease Chemotherapy with Arbotect 20-S® and Alamo®, in: Dunn, C.P. (Ed.), The Elms. Springer, Boston, MA, pp. 173–188. https://doi.org/10.1007/978-1-4615-4507-1_10

Stipes, R.J., Campana, R.J. 1981. Compendium of elm diseases, American P. ed, Compendium of elm diseases. American Phytopathological Society., St. Paul, Minnesota.

Stukenbrock, E.H. 2016. The Role of Hybridization in the Evolution and Emergence of New Fungal Plant Pathogens. Phytopathology 106, 104–112. https://doi.org/10.1094/PHYTO-08-15-0184-RVW

Sturrock, R.N. 2012. Climate change and forest diseases. For. Syst. 21, 329–336. https://doi.org/10.1111/j.1365-3059.2010.02406.x

Süda, I. 2006. Scolytus triarmatus (Eggers, 1912) - New bark beetle in the Baltics. For. Stud. | Metsanduslikud Uurim. 44, 112–117.

Süda, I. 2011. Mardikauurimisest viimastel aastakümnetel (1990–2009) (Coleoptera studies in recent decades (1990−2009)), in: Kull, T., Liira, J., Sammul, M. (Eds.), Eesti Looduseuurijate Seltsi Aastaraamat (Year-Book of the Estonian Naturalists' Society). Eesti Looduseuurijate Selts, Tartu, p. 225−229. [In Estonian]

Suneson, R.B. 2020. Elm disease in the city of Malmö A retrospective (Almsjukan i Mamö, En tillbakablick). Sveriges lantbruksuniversitet. [In Swedish]

Sutherland, M.L., Mittempergher, L., Brasier, C.M. 1995. Control of Dutch elm disease by induced host resistance. For. Pathol. 25, 307–315. https://doi.org/10.1111/j.1439-0329.1995.tb01346.x

Swinton, J., Gilligan, C.A. 1999. Selecting hyperparasites for biocontrol of Dutch elm disease. Proc. R. Soc. B Biol. Sci. 266, 437–445. https://doi.org/10.1098/rspb.1999.0657

Tedersoo, L., Anslan, S. 2019. Towards PacBio-based pan-eukaryote metabarcoding using full-length ITS sequences. Environ. Microbiol. Rep. 11, 659–668. https://doi.org/10.1111/1758-2229.12776

Tedersoo, L., Bahram, M., Põlme, S., Kõljalg, U., Yorou, N.S., Wijesundera, et al. 2014. Global diversity and geography of soil fungi. Science (80-.). 346. https://doi.org/10.1126/science.1256688

Tedersoo, L., Drenkhan, R., Anslan, S., Morales-Rodriguez, C., Cleary, M. 2019. High-throughput identification and diagnostics of pathogens and pests: Overview and practical recommendations. Mol. Ecol. Resour. 19, 47–76. https://doi.org/10.1111/1755-0998.12959

Telnov, D. 2004. Check-List of Latvian Beetles (Insecta: Coleoptera), 2nd ed, Compendium of Latvian Coleoptera. Entomological Society of Latvia.

Thor, G., Johansson, P., Jönsson, M.T. 2010. Lichen diversity and red-listed lichen species relationships with tree species and diameter in wooded meadows. Biodivers. Conserv. 19, 2307–2328. https://doi.org/10.1007/s10531-010-9843-8

Townsend, A.M. 2000. USDA Genetic Research on Elms, in: The Elms. Springer US, pp. 271–278. https://doi.org/10.1007/978-1-4615-4507-1_17

Townsend, A.M., Douglass, L.W. 2004. Evalutation of elm clones for tolerance to Dutch elm disease. J. Arboric. 30, 179–184.

Trubacheva, T., Tsymbal, G., Pimenov, K. 2014. Boulevards of the Historical Center of St. Petersburg (Bulvary istoricheskogo tsentra Sankt-Peterburga) [In Russian]. Mikhailovsky Pushkinian 62, 137–145.

Tziros, G.T., Nakopoulou, Z.G., Perlerou, C., Diamandis, S. 2017. Current status of the Dutch elm disease pathogen populations affecting *Ulmus minor* in Greece. For. Pathol. 47, e12323. https://doi.org/10.1111/efp.12323

Vander Mijnsbrugge, K., Broeck, V., Van, J., Broeck, A. Vanden, Slycken, J. Van 2005. A survey of *Ulmus laevis* in Flanders (northern Belgium). Belgian J. Bot. 138, 199–204.

Venturas, M., Fuentes-Utrilla, P., López, R., Perea, R., Fernández, V., Gascó, et al. 2014a. Ulmus laevis in the iberian peninsula: A review of its ecology and conservation. IForest 8, 135–142. https://doi.org/10.3832/ifor1201-008

Venturas, M., López, R., Gascó, A., Gil, L. 2013. Hydraulic properties of European elms: Xylem safety-efficiency tradeoff and species distribution in the Iberian Peninsula. Trees - Struct. Funct. 27, 1691–1701. https://doi.org/10.1007/S00468-013-0916-7

Venturas, M., Nanos, N., Gil, L. 2014b. The reproductive ecology of Ulmus laevis Pallas in a transformed habitat. For. Ecol. Manage. 312, 170–178. https://doi.org/10.1016/j.foreco.2013.10.007

Voeten, J., Postma, J., Cornelissen, B., Houterman, P., Geijn, H.G. de, Goossen-van de Geijn, H. 2009. Biological control of Dutch elm disease. Gewasbescherming (Plant Prot. 40.

Voolma, K., Mandelshtam, M.Y., Shcherbakov, A.N., Yakovlev, E.B., Õunap, H., Süda, I., Popovichev, B.G., Sharapa, T. V., Galasjeva, T. V., Khairetdinov, R.R., Lipatkin, V.A., Mozolevskaya, E.G. 2004. Distribution and spread of bark beetles (Coleoptera: Scolytidae) around the Gulf of Finland: a comparative study with notes on rare species of Estonia, Finland and North-Western Russia. Entomol. Fenn. 15, 198–210.

Voolma, K., Õunap, H., Süda, I. 2000. Eesti putukate levikuatlas, 2: Ürasklased – Scolytidae (Distribution maps of Estonian insects, 2: Scolytidae). Eesti Loodusfoto, Tartu. [In Estonian]

Voolma, K., Õunap, H., Süda, I. 1997. A key of Estonian bark beetles (Coleoptera, Scolytidae) (Eesti ürasklaste (Coleoptera, Scolytidae) määraja). Eesti Loodusfoto, Tartu. [In Estonian]

Vose, R.S., Easterling, D.R., Gleason, B. 2005. Maximum and minimum temperature trends for the globe: An update through 2004. Geophys. Res. Lett. 32, 1–5. https://doi.org/10.1029/2005GL024379

Waller, M. 2013. Drought, disease, defoliation and death: forest pathogens as agents of past vegetation change. J. Quat. Sci. 28, 336–342. https://doi.org/10.1002/jqs.2631

Webber, J.F., 1987. Influence of the d2 factor on survival and infection by the Dutch elm disease pathogen *Ophiostoma ulmi*. Plant Pathol. 36, 531–538. https://doi.org/10.1111/j.1365-3059.1987.tb02270.x

Webber, J.F. 1990. Relative effectiveness of *Scolytus scolytus*, *S. multistriatus* and *S. kirschi* as vectors of Dutch elm disease. For. Pathol. 20, 184–192. https://doi.org/10.1111/j.1439-0329.1990.tb01129.x

Webber, J.F. 2004. Experimental studies on factors influencing the transmission of Dutch elm disease. Invest Agrar Sist Recur 13, 197–205.

Webber, J.F., Brasier, C.M., 1984. The transmission of Dutch elm disease: a study of the process involved., in: Invertebrate-Microbial Interactions. Cambridge University Press, pp. 271–306.

White, T.J., Bruns, T.D., Lee, S., Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics, in: Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J. (Eds.), PCR - Protocols and Applications - A Laboratory Manual. Academic Press, New York, pp. 315–322.

Whiteley, R. 2004. Quantitative and molecular genetic variation in Ulmus laevis Pall. Swedish University of Agricultural Sciences.

Wingfield, M.J., Brockerhoff, E.G., Wingfield, B.D., Slippers, B. 2015. Planted forest health: The need for a global strategy. Science (80-.). 349, 832–836. https://doi.org/10.1126/science.aac6674

Wingfield, M.J., Garnas, J.R., Hajek, A., Hurley, B.P., de Beer, Z.W., Taerum, S.J. 2016. Novel and co-evolved associations between insects and microorganisms as drivers of forest pestilence. Biol. Invasions 18, 1045–1056. https://doi.org/10.1007/s10530-016-1084-7

Wolfenbarger, D.O., Jones, T.H. 1943. Intensity of Attacks by *Scolytus multistriatus* at Distances from Dispersion and Convergence Points. J. Econ. Entomol. 36, 399–402. https://doi.org/10.1093/jee/36.3.399

Wollerman, E.H. 1979. Attraction of European elm bark beetles, Scolytus multistriatus, to pheromone-baited traps. J. Chem. Ecol. 5, 781–793. https://doi.org/10.1007/BF00986563

Yanovskiy, V.M. 1999. Annotated list of bark beetles (Scolytidae) of North Asia (Annotirovannyy spisok koroyedov (Scolytidae) Severnoy Azii). Entomol. Rev. (Entomologicheskoye Obozr. 78, 327–362. [In Russian]

Zalapa, J.E., Brunet, J., Guries, R.P. 2008. Genetic diversity and relationships among Dutch elm disease tolerant *Ulmus pumila* L. accessions from China. Genome 51, 492–500. https://doi.org/10.1139/G08-034

SUMMARY IN ESTONIAN

JALAKASURMA TEKITAJATE LEVIK JA KAHJUSTUSED KIRDE-EUROOPAS

Sissejuhatus

Jalakaid (*Ulmus* spp.) – ökoloogiliselt ja kultuuriliselt väärtuslikke metsa- või haljastuspuid on jalakasurm, peamiselt põhjapoolkeral, laastanud enam kui sajandi jooksul. Kahe pandeemia ajal on haigus üle ilma hävitanud miljardeid jalakaid. 20. sajandi alguses hukkus hinnanguliselt 10–40% puudest (Peace 1960; Gibbs 1978; Brasier 1996a; Brasier 2000a). Seejärel, 20. sajandi teisel poolel kulgenud teise pandeemia ajal jõudis juba enne sajandivahetust hävida vanematest jalakatest ainuüksi Suurbritannias umbes 80–90% (s.o arvuliselt 28 miljonit) (Brasier and Buck 2001; Kirisits 2013) ning Põhja-Ameerikas veel sadu miljoneid (Brasier 2001; Brasier and Buck 2001). Ameerika Ühendriikides on aastas haigete jalakate raiumisele kulutatud 100 miljonit dollarit (Campbell and Schlarbaum 1994), haiguse kontrolli all hoidmise kulud on samas suurusjärgus (Pimentel et al. 2005).

Esimese pandeemia ajal põhjustas jalakasurma *Ophiostoma ulmi* (Buisman) Nannf. Teise pandeemia ajal on patogeenideks uue invasiivse seene *Ophiostoma novo-ulmi* Brasier kaks alamliiki – subsp. *novo-ulmi* ja subsp. *americana* (Brasier and Kirk 2001; Brasier et al. 2004) ning viimasel ajal on selgunud, et ka nende alamliikide hübriidid (Konrad et al. 2002; Brasier and Kirk 2010).

Jalakasurm on nakatanud jalakaid mitmel pool Euroopas (Clinton and McCormick 1936; Schmidt 2006). Ida-Euroopas on seda haigust määratud Balti riikides (Motiejūnaitė et al. 2016; Jürisoo et al. 2019; Matisone et al. 2020; Jürisoo et al. 2021a, 2021c), Venemaal (Brasier and Kirk 2001), Tšehhi Vabariigis (Dvořák et al. 2007), Poolas (Brasier et al. 2004; Łakomy et al. 2016), Sloveenias (Ogris 2018) ja Horvaatias (Stančin 2018). Soomes märgiti jalakasurma (*O. ulmi*) eelmise sajandi keskel (1952–1968) (Hintikka 1974), kuid uuemate andmete kohaselt pole seda haigust Soomes enam tuvastatud (Hannunen and Marinova-Todorova 2016; 'EPPO Global Database' 2019; Hantula 2021). Jalakasurm oli Lõuna-Rootsis perioodil 1970–2011 vähendanud jalaka populatsiooni

suurust kahe kolmandiku võrra (Brunet et al. 2014) ning aastaks 2020 oli populatsioon kahanenud veel poole võrra (Ruks 2020). Alates 1985. aastast on Rootsis Malmös raiutud üle 40 000 jalaka, mis moodustas 25% kõigist linnapuudest (Suneson, 2020). Rootsis on jalakasurma tõttu kohalike jalaka liikide (*U. glabra*, *U. laevis*, *U. minor*) arvukus vähenenud sedavõrd, et need on võetud punasesse nimistusse (Barstow and Harvey-Brown 2017a, b; Barstow, Rivers and Harvey-Brown 2017). Norras täheldati jalakasurma Oslo lähistel 1963. aastal, tekitajaks *O. ulmi*, üksikuid suuremaid haiguspuhanguid registreeriti veel mujalgi riigis (Gibbs 1978). Mõnikümmend aastat hiljem sai kinnituse *O. novo-ulmi* subsp. *americana* leid, kuid haiguse epideemia Norras siiski taandus (Solheim et al. 2011).

Jalakate tervislik seisund halvenes uuesti oluliselt nii Tallinnas kui mujal Eestis alates 2013. aastast (Jürisoo et al. 2019). Peterburis aga registreeriti jalakasurma juba 2002. aastal umbes 30 haljasalal (sh pargid, alleed), 2017. aastaks oli selliseid haljasalasid juba 50 korda rohkem (Selikhovkin et al. 2010; Shcherbakova et al. 2019).

Paljude invasiivsete haigusetekitajate leviku põhjuseks ja initsiaatoriks on nii globaalne kaubandus kui kliima soojenemine, tõenäoliselt soosivad samad üleilmsed muutused ka jalaksurma levikut. Kohalikus mastaabis on jalakasurma peamisteks kandjateks ehk vektoriteks putukad, eelkõige maltsaüraskid (*Scolytus*), nendegi levikut on suurendanud kliimanihked.

Jalakate seisundi, jalakasurma tekitajate ja selle vektorputukate kohta Eestis ja Loode-Venemaal oli vähe teada. Doktoritöö peamine eesmärk on põhjalikumalt analüüsida jalakate seisundit ja neid laastava haiguse tekitajaid.

Ophiostoma ulmi sensu lato liikidel pole võimalik väliste tunnuste põhjal vahet teha, kuid neid on oluline täpselt määrata, sest erinevatel haiguse tekitajatel on erinev patogeensus ja ohtlikkus puudele (King 2019). Samas on oluline uurida ka peremeestaimede haiguskindlust. Doktoritöö aluseks on neli artiklit, milles kirjeldati jalakate tervislikku seisundit, jalakasurma tekitajaid ja nende levikut ning jalakasurma vektorputukaid.

Doktoritöö hüpoteesid:

1. Jalakasurma tekitaja *Ophiostoma ulmi* on Kirde-Euroopas asendunud uue ja invasiivse patogeeniga *Ophiostoma novo-ulmi*.
2. *Ophiostoma novo-ulmi* on Kirde-Euroopas esindatud kahe teadaoleva patogeeni alamliigi ja nende hübriididega ning nende patogeensus on erinev maapiirkonnas (k.a metsas) ja linnas.
3. Kahe *Ophiostoma novo-ulmi* alamliigi hübriididel on puhaskultuuris (*in vitro*) suurem kasvukiirus kui puhtal alamliigil.
4. Hübriidjalakate suremus on madalam kui looduslikel jalakaliikidel, kuid künnapuu (*Ulmus laevis*) tervislik seisund on Kirde-Euroopa tingimustes parem kui harilikul jalakal (*Ulmus glabra*).
5. Maltsaüraskid (*Scolytus* spp.) on tuntud vektorid jalakasurma levitajana, kuid potentsiaalselt võib vektorliike olla rohkem.

Doktoritöö eesmärgid:

1. selgitada välja jalakaliikide tervislik seisund erinevates kasvukohtades Kirde-Euroopa tingimustes (**I, II**)
2. hinnata jalakasurma levikut Eestis ja Venemaa loodeosas ning isoleerida ja tuvastada seal jalakasurma tekitajad (**I, II, IV**);
3. hinnata ja võrdlevalt analüüsida kahel erineval kalendriaastal jalakate tervislikku seisundit *Ophiostoma novo-ulmi* kahest alamliigist kahjustatud alal (**I**);
4. võrrelda erinevate jalakasurma tekitajate kasvukiirust puhaskultuuris *in vitro* (**II**);
5. koguda, määrata ja analüüsida jalakasurma potentsiaalseid vektorputukaid (**I, II, III**).

Materjal ja metoodika

Kõik uuritud jalakad kaardistati, määrati nende liigid ja hinnati puude tervislikku seisundit nii Eestis kui Loode-Venemaal. Eestis hinnatud hübriidjalakate sordid on teada. Kindlaid hübriidjalakaid Venemaal ei tuvastatud, kuivõrd see on võimalik ainult DNA analüüside põhjal. Jalakatelt, millel olid tüüpilised jalakasurmale viitavad haiguse sümptomid, s.o lehtede närbumine, kolletumine ja kuivamine, koguti laborianalüüside jaoks proovid (**I, II, IV**).

Erinevad jalakate maltsaüraskid (*Scolytus* sp.) määrati igal hinnatud puu tüvel kuni kahe meetri kõrguseni, märkides ära putuka sissepääsuavade ja vastsete galeriide esinemine (**I, II**). 2019. aastal püüti vektorputukad feromoonpüünistega, mis paigaldati erinevatesse asukohtadesse, 23 Tallinna ja 16 asukohta mujal Eestis (kokku 39 tk), lisaks koguti nakatunud puudelt putukaid käsitsi (**III**).

Jalaka liikide tervislikku seisundit uuriti mõlemas riigis – Eestis ja Loode-Venemaal. Puude tervisliku seisundi klasse (vt joonis 3) määrati visuaalse hindamise teel järgmiselt: terve (võras pole nähtavalt närbumist); mõned surnud oksad (mitu oksa surnud ja/või kuni veerandil võrast on lehed närbunud ja puud kuivanud okstega); kahjustatud (palju surnud oksi ja/või kuni pool võrast surnud); suremas (vähem kui pool võrast elus) ja surnud puud (elusaid oksi pole) (**I, II, IV**).

Hariliku jalaka (*U. glabra*) tervislikku seisundit hinnati Eestis samadel aladel 2014. ja 2016. aasta suvel. Umbes 10 ha suurused alad A1 ja B1 valiti patogeenide alamliikide esinemise järgi, et analüüsida patogeenide mõju peremeestaimedele nende kasvukohtades (**I**).

Jalakasurma sümptomitega puudelt võeti proovid ning neist isoleeriti patogeen puhaskultuuri (**I, II, IV**). Kogutud putukate liigid määrati mikroskoobi abil, kasutades erinevaid määrajaid (**III**).

Puhaskultuuridest tuvastati jalakasurma tekitaja liik (*Ophiostoma* sp.) kasutades liigipõhiseid ITS- PCR praimereid, patogeeni alamliigi (subsp. *novo-ulmi* või *americana*) tuvastamiseks kasutati *col*1 ja *cu* geeni spetsiifilisi praimereid, *cu* geeni restriktsioonanalüüsiga ensüümi *Hph*I abil tuvastati alamliikidevahelisi hübriide. PCR-produktid visualiseeriti 1% agaroosi (SeaKem® LE Agarose, Lonza) geelil UV-valguses, kasutades Quantum ST4 (VilberLourmat SAS, Marne-la-allée, Prantsusmaa). Eesti Biokeskuses, Tartus sekveneeriti isolaatide PCR-produktid, mida kontrolliti programmi BioEdit abil (**I, II, IV**). Putukatelt seene liikide tuvastamiseks kasutati kolmanda põlvkonna sekveneerimist (Pacific Biosciences: hiljem PacBio), mis teostati Norras, Oslo ülikoolis (**III**). Tuvastatud liikide kohta uuringutes saadud andmed on leitavad Geenipangas (https://www.ncbi.nlm.nih.gov/), UNITE v.7 andmebaasis (https://unite.ut.ee/) ja Eesti rahvuslikus seenekogus (https://plutof.ut.ee/) (**I, II, III, IV**).

Ophiostoma novo-ulmi mõlema alamliigi ja nende kahe erineva hübriidi (kõigil 3 erinevat isolaati, kokku 12 isolaati) kultuuridega tehtud katse eesmärgiks oli seenemütseeli kasvukiiruse hindamine puhaskultuuris, sentimeetrites ööpäevas. Selle järgi hinnati patogeeni võimalikku agressiivsust – mida kiirem kasv, seda agressiivsem. Mõõtmised tehti neljandal, seitsmendal ja üheteistkümnendal päeval, mõõtes seenekoloonia serva kaugust esialgsest inokulumist (**III**).

Statistiliselt analüüsiti peremeestaimede tervislikku seisundit ja hinnati kasvukohtade (maapiirkondade ja linnapiirkondade) võimalikku mõju jalakate tervisele. Alamseirekohtades A1 ja B1 hinnati jalakate tervisliku seisundi muutumist aastatel 2014 ja 2016, selleks kasutati regrsssioonanalüüsi (**I**).

Erinevate jalakasurma tekitavate patogeenide mõju jalakate tervisele hinnati Mann-Whitney testi abil (**II**). Nelja erineva patogeenivariandi mütseeli kasvukiiruse võrdlevat hindamist puhaskultuuris tehti dispersioonanalüüsi abil (**II**). Jalakasurma tekitaja (*O. novo-ulmi*) osakaalu hindamiseks vektorputukates ning nende seoste uurimiseks erinevate püügimeetodite, proovivõtualade, putukaliikide ja nende sugudega kasutati ANOVA Tukey HSD testi (**III**).

Tulemused ja arutelu

Jalakate tervislik seisund Tallinnas halvenes jalakasurma tõttu alates 2013. aastast, kuid teavet surevate jalakate kohta kogunes mujaltki Eestist, samuti ka Loode-Venemaalt (**I, II**).

Käesolevas töös hinnati Eesti erinevates linna- ja maapiirkondades kokku 2200 jalaka isendit erinevatest liikidest, neist 2014.–2016. aastal 1225 (**I**) ja 2018.–2020. aastal 915 puud (**IV**). Venemaal hinnati 2016. aastal 661 erinevat jalaka isendit. Koguti 626 sümptomaatilist proovi, millest isoleeriti 235 *Ophiostoma* spp. puhaskultuuri (**II**). Seene isolaate analüüsiti mitme PCR praimeriga, mille tulemusena tuvastati esmakordselt Eestis invasiivne patogeen (*Ophiostoma novo-ulmi*) ja Loode-Venemaal. Euroopas jm. esimese epideemia andnud *O. ulmi* esinemist nendes piirkondades ei õnnestunudki meil tuvastada (**I**, **II**, **III**, **IV**).

Ka alad, kus möödunud sajandi esimesel poolel Eestis jalakasurma täheldati, olid enamasti samad, mis käesoleval sajandil, kuid siiski

mõningate erinevustega. Levikukaart (joonis 5) näitab nakatunud puude asukohti Eestis, kus jalakasurma tekitaja tuvastati nüüdseks ka molekulaarselt. Suures osas Eestis on levinud *O. novo-ulmi* subsp. *novo-ulmi,* lisaks sellele on Tallinnas, Loode-Eestis, s.h Hiiumaal levinud Põhja-Ameerika päritolu alamliik (*O. novo-ulmi* subsp. *americana*) (**I, IV**). Haigusetekitaja (*Ophiostoma novo-ulmi* subsp. *americana* x *novo-ulmi*) agressiivse hübriidi esinemine Eestis tõendati esmakordselt 2020. aastal ja seda patogeeni kahe alamliigi levikuala piiril (**IV**).

Venemaa kõigist proovivõtukohtadest leiti *Ophiostoma novo-ulmi* subsp. *novo-ulmi* v.a Peterburi-Moskva kiirtee äärest, kus esinesid vaid alamliikide hübriidid. Venemaa 51 isolaadist määrati 24 isolaati *O. novo-ulmi* subsp. *novo-ulmi* ja viis isolaati subsp. *americana*. Kõik ülejäänud 22 isolaati olid alamliikide vahelised hübriidid – 17 subsp. *novo-ulmi* x subsp. *americana* ja 5 subsp. *americana* x subsp. *novo-ulmi* (**II**).

Ophiostoma novo-ulmi subsp. *americana* osutus agressiivsemaks kui *O. novo-ulmi* subsp. *novo-ulmi*, kuna 28% uuritud harilikest jalakatest (18 tk) alal A1 olid surnud 24 kuu pärast, samas alal B1 olid surnud vaid 4 puud (12%). Regressioonanalüüs näitas, et pärast nakatumist jalakasurmaga (olenemata patogeeni alamliigist) oli keskmine tõenäosus 22%, et jalakad surevad 2 aasta jooksul (**I**).

Lisaks on alamliigi subsp. *americana* kasv *in vitro* subsp. *novo-ulmi* alamliigist pisut kiirem, siiski mitte usaldusväärselt ($P > 0,05$). Patogeeni alamliikide hübriidide kasv puhaskultuuris oli oluliselt ($p < 0,0001$) kiirem kui puhastel alamliikidel, kusjuures kõige kiiremat kasvu näitas *O. novo-ulmi* subsp. *americana* x *novo-ulmi,* mis võib olla teistega võrreldes agressiivsem patogeen (**II**).

Eestis ja Loode-Venemaal on künnapuu (*U. laevis*) tervislik seisund oluliselt parem ($p<0.0001$) kui harilikul jalakal (*U. glabra*). Kaudselt kinnitab seda asjaolu, et iga üheteistkümnes hinnatud harilik jalakas oli jalakasurmaga nakatunud, samas künnapuudest oli nakatunud ainult iga 37s. Jalakate üldine tervislik seisund on jalakasurmast otseses sõltuvuses (**I, II**). Venemaal hinnatud harilikest jalakatest oli halvem tervislik seisund maapiirkonnas, kiirtee ääres. Ilmselt on suure liikluskoormusega aladel puudel suurem stress läbi lokaalse saaste.

Püütud 319 vektorputukast oli võimalikke jalakasurma levitavaid vektorputukaid 261, kellest 81% korjati käsitsi ja 9% püüti feromoonpüünistega. Patogeeni esinemist analüüsiti molekulaarselt kolmanda põlvkonna sekveneermise (PacBio) abil 109 mardikal – nende valik hõlmas erinevaid liike, sugu, asukohti ja erinevaid püügimeetodeid. *Ophiostoma novo-ulmi* leiti seitsmest mardikaliigist kuuel, s.o *Scolytus multistriatus*, *S. triarmatus*, *S. laevis*, *S. scolytus*, *Xyleborinus saxesenii* ja *Xyleborus dispar*. Patogeeni ei leitud ainult *S. pygmaeus*'lt. Käesoleva tööga tuvastati uued jalakasurma tekitaja vektorputukad: *Xyleborinus saxesenii* ja *Xyleborus dispar*, mis näitab selgelt patogeeni ulatuslikumat levikupotentsiaali ja ohtlikkust (**III**).

Olemasolevad nn liigispetsiifilised PCR praimerid suudavad jalaksurma tekitaja tuvastada vaid puhaskultuurist, kuid haiguse kiiremaks äratundmiseks oleks vaja selliseid praimereid, mis suudavad patogeeni ja selle alamliike kindlaks teha ka bioloogilisest proovist, näiteks sümptomaatilisest võrsest eraldatud DNAst. Seni, kuni selliseid molekulaarseid praimerid pole võtta, on võimalik kasutada bioloogilistest proovidest patogeeni tuvastamiseks uue põlvkonna mass-sekveneerimist.

Kokkuvõte ja järeldused

Jalakasurma täpsem uuring Eestis algas 2014. aastal ja Loode-Venemaal 2016. aastal. Aastatel 2014–2020 hinnati ligi 3000 täiskasvanud isendit perekonnast jalakas (*Ulmus*). Koguti üle 600 proovi, millest puhaskultuuri eraldati 235 jalakasurma tekitaja (*Ophiostoma novo-ulmi*) isolaati, nende täpne liik, alamliik ja hübriidid määrati erinevate molekulaarsete praimeritega.

Uuring andis uut teavet jalakasurma, selle haigustekitajate ja vektorite ning jalaka tervisliku seisukorra kohta Kirde-Euroopas. Esmakordselt tuvastati, et nii Eestis kui ka Loode-Venemaal esineb invasiivse patogeeni (*O. novo-ulmi*) kaks alamliiki (subsp. *novo-ulmi* ja subsp. *americana*) ning nende alamliikide vahelised hübriidid.

Analüüsid näitasid, et keskmiselt umbes neljandik jalakasurma nakkusega kohalikest jalakatest (*U. glabra* ja *U. laevis*) sureb tõenäoliselt 24 kuu jooksul. Ameerika alamliik (*O. novo-ulmi* subsp. *americana*) näitas üles suuremat agressiivsust, sest sellega kokkupuutel surid jalakad enam kui kaks korda kiiremini kui Euroopa alamliigiga (*O. novo-ulmi* subsp. *novo-*

ulmi) nakatumisel. Puhaskultuuris (*in vitro*) läbi viidud katse käigus kasvas ameerika päritolu alamliik kiiremini kui kohalik alamliik, kuigi erinevus seente kasvukiirustes kultuuris ei olnud statistiliselt oluline. Patogeeni alamliikide hübriidid aga näitasid puhaskultuuris oluliselt kiiremat kasvu, mis võib viidata suueremale agressiivsusele puudel.

Tulemustest järeldub, et *O. novo-ulmi* subsp. *novo-ulmi* on Eestis laialt levinud; kuid *O. novo-ulmi* subsp. *americana* avastati ainult Loode-Eestist. Samas on mõlemad alamliigid ja nende hübriidid levinud kogu Peterburi piirkonnas. Tulemused näitavad, et jalakate tervislik olukord on Loode-Venemaal erakordselt murettekitav, kuna seal on levinud patogeeni Ameerika tüvi ning Euroopa ja Ameerika tüvede agressiivsed hübriidid. Seega on oht ka Eesti jalakate populatsioonile äärmiselt kõrge.

Eestis on künnapuu (*U. laevis*) tervislik seisund üldiselt parem kui harilikul jalakal (*U. glabra*), mida kinnitab ka puude tervisliku seisundi võrdlev analüüs 2014. ja 2016. aastal. Sarnase kokkuvõtteni jõuti ka Loode-Venemaal (Peterburis).

Selle töö tulemused näitavad, et lisaks teadaolevatele jalakasurma levitavatele jalaka maltsaüraskitele (*Scolytus laevis*, *S. multistriatus*, *S. triarmatus* ja *S. scolytus*), on veel kaks mardikaliiki, kes jalakasurma edasi kannavad: *Xyleborus dispar* ja *Xyleborinus saxesenii*. See tõestab patogeeni oluliselt suuremat levikuvõimet ja ohtlikkust.

Esimene püstitatud hüpotees leidis kinnitust: jalakasurma tekitaja *Ophiostoma ulmi* on Eestis ja Venemaa loodeosas asendunud uue invasiivse patogeeniga *Ophiostoma novo-ulmi* (**I, II, III, IV**).

Kinnitust leidis ka teine hüpotees: *Ophiostoma novo-ulmi* on meie regioonis esindatud kahe teadaoleva alamliigiga ja esineb ka nendevahelisi hübriide (**I, II, IV**). Ameerika päritolu patogeeni patogeensus on erinev võrreldes Euroopa päritolu alamliigiga, mida näitas jalakate tervisliku seisundi hinnang Eesti kahes erinevas proovikohas (**I**). Hariliku jalaka ja künnapuu tervislik seisund Eestis ei sõltunud kasvukohast (**I**). Venemaal oli hariliku jalaka seisund linnapiirkonnas oluliselt parem kui maapiirkondades, kuid künnapuu tervis kasvukohast ei sõltunud (**II**).

Kolmas hüpotees sai kinnitust, kuna patogeeni hübriidid kasvavad puhaskultuuris märksa kiiremini, mistõttu võib alamliikide hübriidide

agressiivsus olla suurem (**II**). Seda hüpoteesi toetab asjaolu, et patogeeni hübriidide laialdane esinemine selgitab ka Loode-Venemaa jalakate oluliselt kehvemat tervislikku seisu.

Neljas hüpotees sai osaliselt kinnitatud, kuna hübriidjalakate suremus oli madalam kui harilikul jalakal, kuid siiski sarnane põlisliigi künnapuuga. Kuid kahest jalaka perekonna põlisliigist on künnapuude tervis oluliselt parem (**I, II**).

Viies hüpotees leidis kinnitust, sest uuringu tulemusel tunnistati Euroopa uuteks jalakasurma levitatavateks vektorputukateks *Xyleborinus saxesenii* ja *Xyleborus dispar* (**IV**).

Doktoritööst tulenevad praktilised soovitused

Tulemused näitavad, et jalakate olukord Eestis ja Loode-Venemaal on erakordselt murettekitav ja tuleb välja töötada tulevikuplaan, kuidas elujõulisi jalakapopulatsioone selles piirkonnas säilitada. Selleks pakume järgmised põhimõtted:

1. Jalakaid tuleb süsteemselt monitoorida vähemalt kord aastas, eelistatavalt suvel (juulis ja augustis), nii metsas kui linnas.
2. Jalakasurma levikut saab aeglustada sanitaarraietega. Avastatud haiged puud tuleb kasvuperioodil eemaldada hiljemalt ühe kuu jooksul nende avastamisest (Haugen 1998). Kui haige puu leitakse suve lõpus, siis võib langetada puhkeperioodil enne aprillikuud, sest sel juhul pole vektorputukad pärast talvitumist veel puutüvest lahkunud ja haigust levitama hakanud. Haiguskolletest ei tohi jalaka puitu mujale transportida (Solheim et al. 2011), äärmisel juhul vaid kinnistes konteinerites ja kooritud puiduna (Jürisoo et al. 2021a). Kindlam oleks puit ja oksad põletada, teha küttepuuks või hakkepuiduks (Liberato et al. 2016). Kännud tuleb juurida. Kui see pole võimalik, siis koorida või freesida (Sandoval et al. 2016) ja katta mullakihiga. Äärmisel juhul võib puutüved jätta alles, kuid need tuleb kindlasti koorida, et piirata haigust levitavate vektorputukate elukeskkonda (Stipes and Campana 1981).
3. Putukate massväljapüüdmine feromoonpüünistega võib olla üks võimalusi jalaksurma tõrjel (El-Sayed et al. 2006). Kuid jalakasurma levitavate putukate masspüüdmiseks Põhja-Euroopas sobivate

feromoonide ja püüniste väljatöötamiseks on vaja täiendavaid analüüse.

4. Käesoleva töö tulemusel saab linnahaljastusse ja metsa soovitada kodumaiste künnapuude istutamist, sest need haigestuvad jalakasurmast harvemini.
5. Enne haljasaladel uute jalakasortide kasutamist on soovitatav katsetada nende sobivust kohalike tingimustega, samuti tuleks eelnevalt testida kohalikku päritolu hariliku jalaka järglaste haiguskindlust.
6. Oluline on järgida põhimõtet, et juhuslike ja tundmatute taimedega koos ei tarnitaks Eestisse ohtlikke patogeene – see aitaks vähendada ka jalakasurma riski. Imporditud istutus- ja puitmaterjal peaks olema kontrollitud ja sertifitseeritud.
7. Käesolevas töös täheldatud jalakate tervislikku seisundit arvestades on väga oluline tuvastada kiirelt ja täpselt jalakasurma tekitaja alamliigid ja nende hübriidid, kuna nende agressiivsus on puudele erinev ja nendest sõltub jalakate tulevik. Selleks on vaja uusi ja usaldusväärseid liigispetsiifilisi DNA praimereid, et bioloogilistest proovidest kiiresti ja täpselt patogeenide alamliike tuvastada. Uue põlvkonna mass-sekveneermine on küll üks võimalus bioloogilistes proovides jalaksurma tuvastamiseks, kuid see ei aita määrata patogeeni alamliike ega hübriide.
8. Kui avastatakse jalaksurma tekitaja ohtlikumad variandid, tuleb nendega nakatunud puud kiiresti ja esmajärjekorras eemaldada, vastasel juhul levivad patogeeni tüved laiemale alale ning suurendavad oluliselt ohtu kogu jalakapopulatsiooni säilimisele.
9. Ohtlike haigustekitajate leviku ohjeldamiseks vajaliku haigete puude raie põhjendamiseks on vajalik teha avalikkuse harimist ja ulatuslikumat selgitustööd.

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I

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Health of elms and Dutch elm disease in Estonia

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Abstract During three years, 2014–2016, Dutch elm disease (further DED) was investigated on 1225 elm trees at 4 different sampling sites and 2 sub-sites in Estonia. For the first time, both subspecies of the invasive pathogen *Ophiostoma novo-ulmi*: *O. novo-ulmi* subsp. *novo-ulmi*, and *O. novo-ulmi* subsp. *americana*, were detected by *col1* and *cu* genes in Estonia and north-eastern Europe. *Ophiostoma novo-ulmi* subsp. *americana* was identified only at one site in northern Estonia, in Tallinn. In addition, during our assessments, the health of elms there appeared worse than at other sampling sites: *O. novo-ulmi* subsp. *americana* demonstrated higher aggressiveness. Simultaneous occurrence of both subspecies and their hybrids was not detected. A repeat survey of 109 elms in 2014 and 2016 demonstrated ca. 22% probability of mortality within 24 months, irrespective of urban vs. rural habitat. In sub-site A1 in Tallinn, *O. novo-ulmi* subsp. *americana* has been found since 2013. DED signs were noted on 39% of all 1225 surveyed trees. Among the assessed elm species, *Ulmus laevis* showed higher resistance than *U. glabra*: 82% and 66% of trees, respectively, showed high vitality. In addition, no *U. laevis* trees were found dead, compared to 18% of the *U. glabra*.

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Introduction

Changing climatic conditions (incl. warmer winters) and global trade have aggravated the invasion of plant pathogens (Brasier 2008; Dehnen-Schmutz et al. 2010; Rytkönen et al. 2008, 2011; Müller et al. 2016; Liebhold et al. 2017; Ghelardini et al. 2017). Several invasive pathogens, e.g., *Hymenoscyphus fraxineus, Dothistroma septosporum, Lecanosticta acicola, Diplodia sapinea,* have invaded Estonia, as expected, the result of climate change and international trade (Hanso and Drenkhan 2009, 2013; Drenkhan et al. 2014, 2015, 2016; Adamson et al. 2015a, b, 2018a, b).

The health status of elms in Tallinn, northern Estonia, worsened substantially in 2013. DED was confirmed as the cause (R. Drenkhan, pers. comm.). It is possible that the threat to *Ulmus* spp. has risen in Estonia due to the trade of infected elm plants or wood as shown by La Porta et al. (2008) and Solheim et al. (2011) in other countries, similar to what has happened in Sweden, Norway, the UK and USA (Brasier and Kirk 2010; Solheim et al. 2011; Menkis et al. 2016). Mortality due to DED has decimated elm populations, like in Sweden, where all the native elm species (*U. glabra*, *U. laevis*, *U. minor*) are on the Red List as critically endangered (Barstow and Rivers 2017; Barstow and Harvey-Brown 2017; Barstow

et al. 2017). The DED agent is known to kill trees rapidly (Phillips and Burdekin 1992; Schmidt 2006).

In the forests of Estonia, two native elm species, *U. glabra* and *U. laevis*, occur rarely – forming only 0.1% of the total volume of forest trees (Raudsaar et al. 2016). *Ulmus glabra* can be found throughout Estonia, while *U. laevis* is much rarer and is growing mainly along riversides (Kukk and Kull 2005). *Ulmus laevis* is listed as "near threatened" in Estonia according to the IUCN Red List of Threatened Species (Lilleleht 2008; IUCN 2018; Leht 2018); thus, the value of the tree species is even higher because of ecological and cultural reasons (Martín et al. 2018). Elms are common and valuable amenity trees in urban spaces (Aaspõllu 1999; Kaar 2011) and in rural areas (incl. historical parks and forests) all over Estonia (Abner et al. 2007, 2012). Some of these historical parks had been formed from boreo-nemoral forests (Kalda 1995; Tamm 2007), which are considered good environments for broad-leaved trees such as elms in Estonia (Paal 1998). Groups and even monocultures of elm trees can be found in these parks (Kristian 1939).

Generally, elm is an ecologically important tree genus in the dendroflora of the northern Baltic region. Loss of elms causes a loss of many associated organisms (Thor et al. 2010). For example, 39 different epiphytic lichens, obligate on broadleaved trees, live on *U. glabra* in Estonia, incl. two near-threatened species and one endangered (Jüriado et al. 2009). Two fungi, *Rhodotus palmatus* and *Hymenochaete ulmicola*, obligately associated to elms, are endangered (Corfixen and Parmasto 2005; Kalamees 2011).

Taxonomically and pathologically related species, *Ophiostoma ulmi* (Buisman) Nannf. and *O. novo-ulmi* Brasier, had infected *Ulmus* species in many parts of Europe (Clinton and McCormick 1936; Schmidt 2006). In Estonia, according to the European and Mediterranean Plant Protection Organization (EPPO) global database, *O. ulmi* has been reported since 1979 (EPPO 2017). Actually, *O. ulmi* has been reported in Estonia since the 1930s (Lepik 1940). In 2006, *O. novo-ulmi* was detected for the first time in Estonia (Hanso and Drenkhan 2007), but at that time it was distinguished neither as a subspecies nor as a hybrid. In Europe, DED is caused by two different subspecies of the pathogen – *O. novo-ulmi* subsp. *novo-ulmi* and *O. novo-ulmi* subsp. *americana* (Brasier and Kirk 2001; Brasier et al. 2004; Martín et al. 2010). Information about the occurrence of the subspecies and their hybrids in regions closest to Estonia comes from southern Norway, Sweden (Brasier and Kirk 2010) and Lithuania (Motiejūnaitė et al. 2016).

Mortality by DED varies also depending on the host (*Ulmus*) species, levels of susceptibility and genetic variation (Martín et al. 2018), as well as stand density and possible rootgrafts (Santini and Faccoli 2013) of trees in natural stands or urban areas, but is also influenced by pathogen spore concentration (Flower et al. 2017). In its natural habitats in Poland, for example, *U. minor* suffered more and *U. laevis* less than *U. glabra* (Łakomy et al. 2016). *Ulmus laevis* is considered less susceptible to DED because it is less attractive to the beetles (Collin 2002).

Insect vectors (e.g. *Scolytus* spp.) are essential agents in spreading *O. novo-ulmi*. It is thought that northern Europe is protected from DED because of a lack of these insect vectors (Caulton et al. 1998; La Porta et al. 2008). *Scolytus* beetles of elms have not been found in Finland (Voolma et al. 2004; Hannunen and Marinova-Todorova 2016), but several of them (e.g., *S. scolytus*, *S. laevis*, *S. multistriatus*, *S. triarmatus*) inhabit Estonia, with some of them discovered as early as the first half of the last century (Voolma et al. 2000, 2004).

So far, DED has not been documented in areas closer to Estonia – Finland and north-west Russia, where the natural species composition is the same (Hannunen and Marinova-Todorova 2016; EPPO 2017); therefore, our investigation should represent the northeastern-most survey of DED in Europe.

The aim of the research is to assess the health conditions of elms affected by DED agents to protect local elm populations over the long term.

The specific objectives of this work were: (1) to isolate and identify the causative agents of DED in Estonia, (2) to monitor the health status of elms in different sampling sites and habitats over three consecutive years, and (3) to assess and compare the vitality of elms affected by the two subspecies of *Ophiostoma novo-ulmi* in two non-consecutive years, 2014 and 2016.

Material and methods

Study sites and health survey of elms

The health status of elms was surveyed over three years (2014–2016) at four different sampling sites (incl. two sub-sites – A1 and B1) in Estonia. One sample collected

by R. Drenkhan in 2013 from Tallinn was also included, as it was the key for this research as DED started to spread in the town quite intensively. The sites were selected based on published information regarding the occurrence of elms in Estonia (Kukk and Kull 2005; Saarse and Veski 2001) and dendrofloristic inventories of parks (Laas and Treumuth 2006; Abner et al. 2007, 2012; Rist 2015). Samples were collected in various urban and rural areas. In total, 1225 mature elm trees were assessed. Of them, 109 (*U. glabra*) were assessed twice in summer of 2014 and summer of 2016 in sub-sites A1 and B1. The time between the assessments was 24 months and both sub-sites have an area of ca. 10 ha (see Fig. 1). The sub-sites A1 and B1 were chosen by pathogen sub-species to analyse the effect of the pathogens on hosts under natural conditions. In this study, urban space includes streets, city parks and urban forests. Selected rural habitats were close to roads (avenues) or situated in rural parks (historical manor parks) and forests. More than half (57%) of the surveyed trees (694) were in urban space, while 531 (43%) were at rural sites. A total of 238 symptomatic samples were collected for laboratory analyses during the three years of this survey.

During the survey, eight different elm taxa and at least two cultivars were assessed. 1016 (82.9%) of the investigated trees were *Ulmus glabra*, 181 (14.8%) were *U. laevis* and 28 trees (2.3%) were hybrids or non-native *Ulmus* species (Table 1).

All the mature elm trees growing at a site were mapped, and identified by species and varieties, according to Hillier Nurseries (1991). The variety (planted along the Euroroute R1 in 2015) 'New Horizon' (Johannes Grothaus, pers. comm.) is a hybrid *Ulmus davidiana* var. *japonica* × *U. pumila*. The presence of bark beetles was assessed according

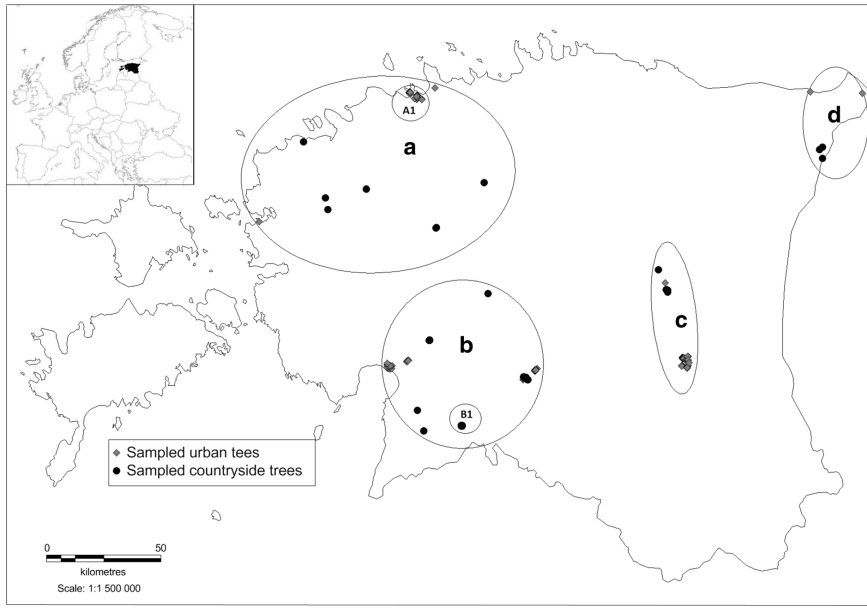


Fig. 1 Locations of the four sampling sites (**a**, **b**, **c**, **d**) in Estonia. The same sample trees (N = 109) were estimated in two non-consecutive years (2014 and 2016) in smaller sub-sites A1, Tallinn and B1, Tihemetsa. At sampling site **a** there are 15 different habitats and 8 of them in Tallinn (in A1–5). At sampling site **b** there are 12 different habitats (incl. B1–1 habitat), at sample site **c** – 10 and at sample site D – 3. DED was isolated from samples collected from sampling sites **a**, **b** and **c**, but not from site **d**

Table 1 Amount of *Ulmus* spp. trees analysed in four sampling sites (see Fig. 1) and habitats

Location	Habitat	Total	Surveyed species			
			Ulmus glabra[a]	*U. laevis*	*U.* hybrid[b]	*U.* spp.[c]
Urban space		694	614	53	20	7
	Park	325	302	21		2
	Street	255	200	30	20	5
Rural		531	402	128		1
	Park	271	225	45		1
	Road avenue	21	5	16		
	Forest	239	172	67		
Number of trees		1225	1016	181	20	8
Total assessed trees (%)		100	82.9	14.8	1.6	0.7

[a] Number of surveyed trees contains different *Ulmus glabra* cultivars: 'Camperdownii' (11),'Exoniensis' (25)

[b] *Ulmus davidiana* var. *japonica* × *U. pumila* 'New Horizon' (20)

[c] Other non-native species: *U. minor* (5), *U. procera* (1), *U. minor* f. *suberosa* (1), *U. pumila* (1)

to Atkinson (2017) and Süda (2006). Symptomatic foliage conditions, incl. wilting, yellowing and browning of leaves, were considered to be caused by DED agent (Solheim et al. 2011).

The survey was carried out from June to October in three consecutive years (2014–2016). Five general crown vitality classes were determined by visual assessment according to Rosenvald et al. (2015) with some original genus-specific modifications and corrections for *Ulmus* spp. (see Fig. 2).

Crown conditions were estimated as: healthy (no visible wilting of leaves in the crown); branch loss (several branches dead and/or up to one-quarter of the crown consisting of wilting of leaves and dry branches); damaged (many dead branches and/or up to half of the crown consisting of wilting of leaves and dead branches); dying (less than a half of live branches remaining) and dead trees (no live branches remaining).

If external disease symptoms were obvious and typical for DED (Santini and Faccoli 2013) and dark brown dots or rings in xylem of symptomatic twigs were confirmed (Łakomy et al. 2016), the samples were taken for laboratory analyses. Twigs or shoots were cut with telescopic secateurs that were sterilized after each cut. Each sample was separately packed into a labelled sterile plastic bag, transported to the lab and stored for a maximum of 1 week at +4 °C until the fungal isolations.

The presence of elm bark beetles was determined on the trunk of every assessed tree at up to 2 m height, noting the occurrence of entrance holes and larval galleries (Santini and Faccoli 2013).

The maps were compiled using MapInfo Professional version 15 (Pitney Bowes Software 2015).

Fig. 2 Crown vitality classes of surveyed *Ulmus* spp. trees (here illustrated by *U. glabra*): 1, healthy; 2, branch loss; 3, damaged; 4, dying; 5, dead

Climate conditions in the years of assessment

Climate data from meteorological stations near the sites (Keskkonnaagentuur 2015, 2016, 2017) during the survey years (2014–2016), along with average values since 1981, are shown in Table 2.

Fungal isolation and DNA extraction

Pathogens and other fungi (Appendix Table 6) were isolated from the symptomatic shoots similar to Drenkhan et al. (2017) with some modifications:

1. Bark of the symptomatic shoots was peeled off with a sterile scalpel and a thin layer of wood was removed up to dark brown rings in xylem. After that, small pieces of the infected wood tissue were placed on sterile MEA (Malt Extract Agar) and incubated at room temperature for 7–14 days.
2. Subcultures were made by transferring small amounts of mycelium from colonies into new plates and incubated for ca. 14 days.
3. Ca. 0.04 g of mycelium taken from the culture was transferred into 2.0 ml micro centrifuge tubes for DNA extraction using a Thermo Scientific GeneJET Genomic DNA Purification Kit (Thermo Fisher Scientific, EU).

DNA was stored at −20 °C until further analyses.

PCR and sequencing

Species-specific PCR primers mtsr1 (5′-AGTG GTGTACAGGTGAG-′3) and mtsr2 (5′-CGAG TGGTTAGTACAATCC-′3) (Gibb and Hausner 2005) were used for quick detection of *O. ulmi* and *O. novo-ulmi* from mycelial DNA. Then subspecies of *Ophiostoma novo-ulmi* were detected from mycelial DNA by the gene *col1* species-specific primed PCR (SSPP) that was performed using the primer pair F-primer (5′-GCAGTTGTTGACATGTATG-′3) and R-primer (5′-TGCTTGACGTAGATCTCG-3′) described by Konrad et al. (2002). The *cu* gene region was amplified with the primers CU1 (5′-GGGCAGCTTACCAG AGTGAAC-3′) and CU2 (5′-GCGTTATGATGTAG CGGTGGC-3′) (Pipe et al. 1997) and then digested by restriction enzyme *Hph* I (New England Biolabs, USA) to also identify subspecies of *Ophiostoma novo-ulmi* (see Konrad et al. 2002; Dvořák et al. 2007, and the manufacturer's instructions). The purpose of analysing the two genes (*col1* and *cu*) of *Ophiostoma novo-ulmi* was to detect hybridization of the pathogen (Dvořák et al. 2007; Tziros et al. 2017).

Detection of *Ophiostoma* sp. and other fungi was performed from DNA extracted from pure cultures using the fungal-specific ITS PCR primers ITS1-F (5′-CTTGGTCATTTAGAGGAAGTAA -3′; Gardes and Bruns 1993) and ITS4 (5′- CCTCCGCTTATTGA TATGC -3′; White et al. 1990) and carried out as described by Drenkhan et al. (2017).

PCR products of randomly chosen samples of all symptomatic hosts from the above-mentioned sampling

Table 2 Minimum, maximum, and mean temperatures and precipitation for the years 2014, 2015, and 2016 and for 1981–2016, including vegetation periods, from two meteorological stations

Sampling sites and meteorological stations	Calendar year	Air temperature (°C)				Precipitation sum (mm)	
		Annual min	Annual max	Annual mean	Vegetation period mean	Vegetation period	Annual
A and Tallinn-Harku	2014	–18.7	31.6	6.8	13.0	328.6	575.9
	2015	–12.3	28.4	7.5	12.3	328.6	590.0
	2016	–20.9	27.7	6.6	13.2	456.2	773.7
	1981–2016			7.5	12.8	393.6	622.8
B and Pärnu-Sauga	2014	–22.7	31.8	7.0	13.5	427.9	740.5
	2015	–14.5	29.3	7.5	12.6	372.5	729.9
	2016	–25.1	29.8	6.7	13.7	425.5	745.7
	1981–2016			7.0	13.6	402.8	773.5

sites were sequenced in order to find the subspecies and spread of their hybrids.

The PCR products were visualized on 1% agarose (SeaKem® LE Agarose, Lonza) gels under UV light using the Quantum ST4-system (VilberLourmat SAS, Marne-la-allée, France). All amplifications were performed at least twice to ensure consistent banding patterns.

Randomly chosen samples from different sites and hosts were sequenced at the Estonian Biocentre in Tartu, using the primers ITS5 (5′-GGAAGTAAAAGTCG TAACAAGG-3′; White et al. 1990), and primers F and R for sequencing of *col1* gene (Konrad et al. 2002). The sequences were edited using the BioEdit program, Version 7.2.5 (Hall 2013) and deposited in GenBank (www.ncbi.nlm.nih.gov/genbank/) (see Table 3). BLAST searches for the fungal taxa confirmation were performed using the GenBank database (https://www.ncbi.nlm.nih.gov/). ITS sequence similarity threshold was ≥99% for *Ophiostoma* spp. and ≥ 97% for other fungal species detection. The *col1* gene sequence similarity threshold was ≥99% for *Ophiostoma novo-ulmi* subspecies detection.

Statistical analyses

Statistical analyses were carried out to evaluate for changes in host vitality, for probable change in tree condition, and for analyses of the impact of habitat (rural versus urban area) on the health of elms.

Two sets of analyses were carried out for estimation of health condition change of elms between 2014 and 2016 in sub-sites A1 and B1 (Fig. 5). In this case, the regression formula (1) was used:

$$\mathrm{Vc}_{2016} = a_0 + a_1 \cdot \mathrm{Vc}_{2014}, \quad (1)$$

where Vc_{2016} represents the vitality class in 2016; Vc_{2014} is the vitality class in 2014, and a_0, a_1 are regression coefficients.

For prognosis of changing tree conditions over 2 years, regression formula (2) was used to guarantee regression pervasion through point (5; 5) (see Fig. 6):

$$\mathrm{Vc}_{2016} - 5 = a \cdot (\mathrm{Vc}_{2014} - 5), \quad (2)$$

where Vc_{2016} is the vitality class in 2016; Vc_{2014} is the vitality class in 2014, and a represents the regression coefficient.

Regression analyses were implemented for sub-sites A1 and B1 together (see Fig. 6) and separately to compare the health conditions (formula 4) of elm trees on these sites.

Location identifiers (sub-sites A1 and B1) were nominal characteristics. The nominal features were changed to numerical. It was assigned one for sub-site A1 (C_A = 1) to calculate this characteristic for sub-site B1 with the next formula (3):

$$C_B = \frac{a_B}{a_A}, \quad (3)$$

where C_B is the numeric value for nominal characteristic "Sub-site B1", and a_B and a_A are constants for regression formula 2 according to the data (sub-site B1 and A1). Values depending on location (C_B and C_A) were added to the data. For the following regression analyses (see Fig. 6) the numeric characteristic of location was added, and the formula was used as follows (4):

$$\mathrm{Vc}_{2016} - 5 = a \cdot (\mathrm{Vc}_{2014} - 5) \cdot C, \quad (4)$$

where Vc_{2016} represents the vitality class in 2016; Vc_{2014} is the vitality class in 2014, C is the characteristic of sub-site A1 or B1, and a is a coefficient.

Regression analyses were used for calculating health conditions in different habitats of native elm species. Exotic species and cultivars were excluded from these calculations.

Results

Dutch elm disease agents at different sampling sites and on different hosts

In total 238 samples were collected, from which 76 pure cultures of *Ophiostoma novo-ulmi* were successfully isolated (see Fungal isolation and DNA extraction in Material and methods). The species-specific PCR primers were used to check all the strains, which were *O. novo-ulmi* (Gibb and Hausner 2005). *Col1* gene sequencing confirmed the first occurrence of both known *O. novo-ulmi* subspecies in Estonia (see Table 3), and *O. ulmi* was not found. *Ophiostoma novo-ulmi* subsp. *novo-ulmi* was found in sampling sites B and C situated in southwest and central Estonia (Fig. 1). *Ophiostoma novo-ulmi* subsp. *americana* was found

Table 3 Origin of isolations from symptomatic samples of different *Ulmus* species and molecularly detected DED agents by gene *col1* and digested gene *cu* with enzyme *Hph* I, accession No in GenBank (NCBI) by *col1* gene and ITS

No	Identification No	Sampling				Host species	Tested isolations of SSPP *Ophiostoma novo-ulmi*	Molecular identification *Ophiostoma novo-ulmi* subsp.		Accession no. GenBank	
		site or sub-site	date	location	habitat			by *col1*	by *cu*+ *Hph* I	by *col1* gene	by ITS
1	1050	A	18.06.2013	urban space	park	*Ulmus glabra*	+	*americana*	*americana*	MF784565	MF754038
2	4651	A	02.07.2015	urban space	park	*U. glabra*	+	*americana*	*americana*		
3	4652	A	02.07.2015	urban space	street	*U. glabra*	+	*americana*	*americana*		
4	6839	A1	14.07.2016	urban space	street	*U. glabra*	+	*americana*	*americana*	MF784568	MF766443
5	6833	A1	14.07.2016	urban space	street	*U. glabra*	+	*americana*	*americana*		
6	6835	A1	14.07.2016	urban space	street	*U. glabra*	+	*americana*	*americana*		
7	6836	A1	14.07.2016	urban space	street	*U. glabra*	+	*americana*	*americana*		
8	6842	A1	14.07.2016	urban space	street	*U. glabra*	+	*americana*	*americana*		
9	6845	A	14.07.2016	urban space	street	*U. glabra*	+	*americana*	*americana*		
10	6880; 6840	A1	14.07.2016	urban space	street	*U. glabra*	+	*americana*	*americana*		
11	8326	A1	14.07.2016	urban space	street	*U. glabra*	+	*americana*	*americana*		
12	4533	B	01.07.2015	urban space	forest	*U. glabra*	+	*novo-ulmi*	*novo-ulmi*	MF784566	MF754039
13	4534	B	01.07.2015	urban space	forest	*U. glabra*	+	*novo-ulmi*	*novo-ulmi*		
14	4618	B	01.07.2015	urban space	forest	*U. glabra*	+	*novo-ulmi*	*novo-ulmi*		
15	4638	B	19.07.2015	rural	park	*U. glabra*	+	*novo-ulmi*	*novo-ulmi*	MF784567	MF766442
16	4751	B	24.08.2015	rural	avenue	*U. laevis*	+	*novo-ulmi*	*novo-ulmi*		
17	6851	B1	11.05.2016	rural	park	*U. glabra* 'Camperdownii'	+	*novo-ulmi*	*novo-ulmi*	MF784570	MF766445
18	6856	B	09.07.2016	urban	park	*U. glabra*	+	*novo-ulmi*	*novo-ulmi*	MF784571	MF766446
19	6963	B	09.07.2016	rural	park	*U. glabra*	+	*novo-ulmi*	*novo-ulmi*		
20	6843	B1	10.07.2016	rural	park	*U. glabra*	+	*novo-ulmi*	*novo-ulmi*		
21	6855	B1	10.07.2016	rural	park	*U. glabra*	+	*novo-ulmi*	*novo-ulmi*		
22	6975	B	20.07.2016	rural	forest	*U. glabra*	+	*novo-ulmi*	*novo-ulmi*		
23	6948	B	20.07.2016	rural	forest	*U. glabra*	+	*novo-ulmi*	*novo-ulmi*	MF784572	MF766447
24	6952	B	20.07.2016	rural	forest	*U. laevis*	+	*novo-ulmi*	*novo-ulmi*	MF784573	MF766448
25	6844	C	03.07.2016	urban	park	*U. glabra*	+	*novo-ulmi*	*novo-ulmi*	MF784569	MF766444

Identification No – in collection of laboratory of forest pathology of Estonian University of Life Sciences

SSPP – species-specific PCR primers (Gibb & Hausner, 2005) were used to detect *Ophiostoma novo-ulmi* or *O. ulmi* for all the isolations

col1 – the colony type gene (Konrad et al. 2002); the sequence similarity threshold ≥ 99% for *Ophiostoma novo-ulmi* subspecies detection

cu+*Hph* I – ceratoulmin gene - RFLP banding pattern of *cu* from *O. novo-ulmi* digested with enzyme *Hph* I (Konrad et al. 2002)

ITS – internal transcribed spacer; the sequence similarity threshold is ≥ 99% for *Ophiostoma* spp. detection

GenBank (NCBI- https://www.ncbi.nlm.nih.gov/)

only in Tallinn, at a location in sub-site A1 (see Fig. 1). *O. novo-ulmi* subsp. *novo-ulmi* was detected on *U. glabra* and its variety 'Camperdownii', and on *U. laevis*. *O. novo-ulmi* subsp. *americana* was found on *U. glabra* in Tallinn (see Table 3). No *O. novo-ulmi* was found in northeast Estonia (sampling site D) – neither in urban spaces nor at rural sites. *Ophiostoma novo-ulmi* hybrids by colony type (*col1*) gene and ceratoulmin gene (*cu*) were not detected at any sampling sites in Estonia (see Table 3).

Insects on assessed trees

All the assessed trees (N = 1225) were examined for signs of elm bark beetles, e.g., entrance holes, larval galleries etc. Thirty randomly selected *U. glabra* sample trees had entrance holes of bark beetles. Larval galleries were found only on dead trees at sample sites A and B, but not at site C. Those galleries belonged to *Scolytus multistriatus*, *S. scolytus* or *S. triarmatus* (K. Voolma, entomologist, pers. comm.). Signs of the latter two species were considered so similar that it was impossible to identify the species without seeing the adult beetles (see Süda 2006).

Health condition of different elm species in Estonia

At the sampling sites, the dieback or death of elm trees was considered to be caused by DED. Among all assessed trees (N = 1225) *U. laevis* showed significantly ($p < 0.001$) higher vitality than *U. glabra*, with 82% of *U. laevis* and only 66% of *U. glabra* trees rated as vitality class 1 and 2. 18% of *U. glabra* trees, but no *U. laevis* trees were found dead (Fig. 3).

Correlation between DED symptoms and vitality class of native elm species (*U. glabra* and *U. laevis*) showed that 29% or 30% of DED-symptomatic trees were in vitality classes 2 and 3, but 80% in class 4 (Fig. 4). This data shows that health of elm species in Estonia (indicated by vitality class) correlates significantly ($p < 0.001$) with the DED symptoms.

Vitality changes of elms in sub-sites A1 and B1 from 2014 to 2016

In this health analysis, we considered trees that were assessed in 2014 and 2016 (N = 109). All these trees were *U. glabra,* 72 at sub-site A1 and 37 at B1. Over this period, the proportion of healthy *Ulmus* spp. fell significantly ($p < 0.001$). In 2014 about 50% of assessed trees were healthy, but in 2016 only five (5%) of the 109 trees investigated were still healthy. Ten sampled trees (9%) were found dead in 2014. In 2016 a total of 32 trees (29%) were dead (Fig. 5).

From 2014 to 2016, the vitality of elms clearly diminished according to the repeated vitality estimation of the same 109 trees in sub-sites A1 and B1. The calculated regression line (formula 2, see the "Statistical analyses" section) shows that during these 24 months the health condition of each sampled tree decreased by ca. 0.77 vitality class (Fig. 5). In 2014 we classified 22 trees (20%) as vitality class 1 to 4, but after 24 months all these trees were dead.

One purpose of this study was to describe the vitality change of elms in this period at two sub-sites (A1 and B1) as *O. novo-ulmi* subsp. *americana* was causing damage in one location (Tallinn) at the sub-site A1, but *O. novo-ulmi* subsp. *novo-ulmi* was causing damage at sub-site B1. The vitality changes between 2014 and 2016 (24 months) of the surveyed trees was calculated by formula 2 (see Fig. 6, for details see Table 4). The effect of the two sub-sites (separately A1 and B1) on the vitality of elms was calculated by regression formulas 3 and 4 (see "Statistical analyses" section). This analysis showed that the vitality of elms was significantly lower ($p < 0.00001$) at sub-site A1 than at sub-site B1 (see Fig. 6). 18 elms (25%) at A1 and 4 elms (12%) at B1 were living in 2014, but dead by 2016.

Taking into account that the assessed DED-infected elms (N = 109) at sub-sites A1 and B1, the probability of dying after 24 months for elms in vitality classes 1–4 was 22%, and in class 4 (already dying trees) 78% (see Table 5). The data did not indicate that the health condition of elm trees improved during the monitoring years (see Figs. 5 and 6).

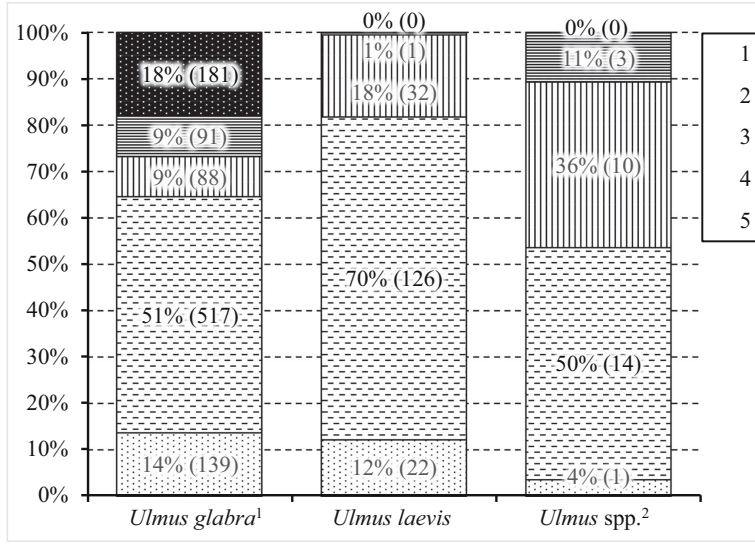
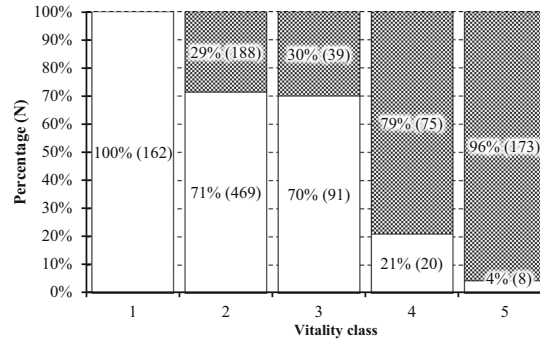


Fig. 3 Health conditions of *U. glabra, U. laevis* and other *Ulmus* spp. based on their representation in vitality classes 1–5, see Fig. 2. The percentage of sampled trees and the number of trees (N) are listed per vitality class. [1]The number of surveyed trees consists mainly of *Ulmus glabra* and its cultivars: 'Camperdownii' (11 trees), 'Exoniensis' (25). [2]Identified exotic *Ulmus* species: *Ulmus davidiana* var. *japonica* × *U. pumila* 'New Horizon' (20), *U. procera* (1), *U. minor* (5), *U. minor* f. *suberosa* (1), *U. pumila* (1)

Fig. 4 The relative incidence of symptoms of DED (white area without symptoms, squared area – with symptoms) on *U. glabra* and *U. laevis* depending on vitality class (see Fig. 2), the percentage of representatives and number of assessed trees (N) in a definite vitality class



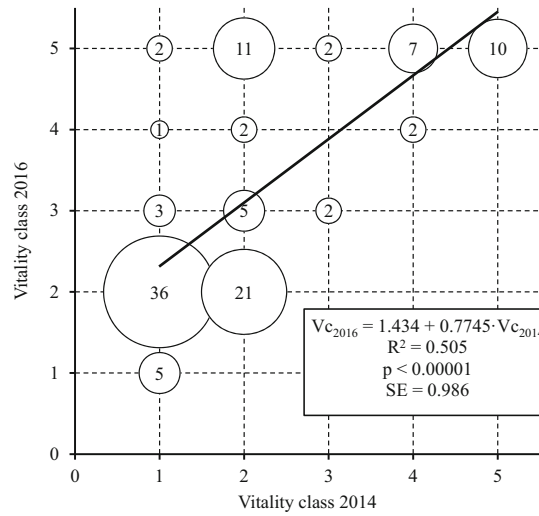


Fig. 5 Change in the vitality of surveyed elms (N = 109) during 24 months (from 2014 summer to 2016 summer). The numbers (in circles) indicate how many trees have kept or lost their vitality. The bigger circles show the higher number of trees in a definite vitality class in 2016. The rising line shows the growing decline of vitality for 109 surveyed elms at two sub-sites (A1 and B1)

Health conditions of elms at two different locations

Of the assessed elm trees, 36% urban sites and 43% of rural sites had symptoms of DED, a statistically insignificant difference ($p > 0.05$). This did not show differences in health conditions of elms at any sampling site. The differences between the meteorological characteristics (mean temperatures and precipitation sums) of the two meteorological stations compared over the surveyed years (2014–2016) were statistically insignificant ($p > 0.05$), and other abiotic reasons were not analysed in this work. Not all isolated fungi (except *Ophiostoma novo-ulmi* subsp.) from elm shoots are known to be pathogens to elms (see Appendix Table 6).

Discussion

Although DED is one of the most investigated diseases from many perspectives, there are still various questions about plant-pathogen interactions (Bernier et al. 2014). In this study, two subspecies of the invasive pathogen – *Ophiostoma novo-ulmi* subsp. *novo-ulmi* and *O. novo-ulmi* subsp. *americana* – were documented for the first time in Estonia and north-eastern Europe. The occurrence of *O. ulmi,* the

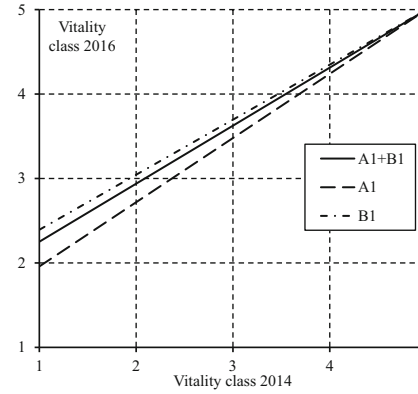


Fig. 6 Probability of changing health of elms at sub-sites A1 + B1 (solid line) together and two sub-sites A1 and B1, separately calculated. The solid line here is from the same data seen in Fig. 5

Table 4 Description of vitality changes of DED-infected elms during 24 months for the trees assessed in 2014 and 2016 at sub-sites A1 and B1

Sub-site	Number of observed trees	a (a·C)	SE	R^2	*p* value
A1 and B1	109	0.70955	0.99	0.505	< 0.0001
A1	72	0.67704	1.03	0.490	< 0.0001
B1	37	0.76344	0.84	0.583	< 0.0001

Parameters of regression analyses (number of observed trees, *a* – regression coefficient) for formula 2 (see Statistical analyses) with standard errors (SE), coefficients of determination (R^2) and significances (*p* value)

previous agent of DED, was not detected during this study. In northern Estonia, only *O. novo-ulmi* subsp. *americana* (11 isolates from randomly chosen symptomatic trees) was found in 5 different habitats in Tallinn, but not at the other locations. It proved to be more aggressive than *O. novo-ulmi* subsp. *novo-ulmi*, as 28% of surveyed elms (18) after 24 months were found dead at sub-site A1. At sub-site B1, where *O. novo-ulmi* subsp. *novo-ulmi* was causing damage, only 4 (12%) were dead. Regression analyses indicated that after being infected by DED (caused by both subspecies), the mean probability of elm trees to die within 2 years was ca. 22% among the 109 elm trees surveyed. Concluding all 1225 elms then *U. laevis* survived better (none of the trees was found dead) than the other naturally occurring *U. glabra*.

If both subspecies occur in the same region, there is a possibility for hybridization between them. No hybrids between *O. novo-ulmi* subspecies were detected in Estonia.

DED in Estonia

DED was diagnosed in Estonia by the 1930's (Lepik 1940). The pathogen was expected to spread across most of the country (Lepik 1940; Kaar 2011). At that time, the pathogen in Europe was known as *O. ulmi* (Brasier and Buck 2001). This agent may leave many infected elm trees alive because it was not so devastating (Kirisits 2013). In the beginning of the twenty-first century, *O. ulmi* has still been found in some parts of Europe (Solla et al. 2008). In neighbour countries

Table 5 The probability of decline over two non-consecutive years 2014 and 2016 (total 24 months) for DED in each health vitality class of elms at sub-sites A1 and B1

	Vitality in year x				
Vitality in year x + 2	Healthy	Branch loss	Damaged	Dying	Dead
Healthy	0.106				
Branch loss	0.767	0.538			
Damaged	0.064	0.128	0.500		
Dying	0.021	0.054	0.000	0.222	
Dead	0.042	0.283	0.500	0.778	1.000
N	47	39	4	9	10

N number of observed trees

DED was still present as *O. ulmi*, e.g. in Latvia (since 2012), Lithuania (since 1979) and in the European part of Russia (since 1979) (EPPO 2017). However, *O. novo-ulmi* is considered to be widespread in southern Russia (Gibbs 1978) and later on *O. novo-ulmi* subsp. *novo-ulmi* was detected there (Brasier and Kirk 2001). There exists a restricted distribution of *O. ulmi* in southern parts of Sweden and Norway (EPPO 2017), whereby *O. novo-ulmi* and the both subspecies having been found as well (Brasier and Kirk 2001). DED (but without molecular identification of its agent) had been found in Finland in 1988 (EPPO 2017), but it was not found there later (Hannunen and Marinova-Todorova 2016).

Ophiostoma ulmi was not identified in Estonia during this investigation, similar to what has been observed in many regions of Europe, where the more aggressive *O. novo-ulmi* displaced the earlier naturally occurring species *O. ulmi* (Brasier and Buck 2001; Brasier and Kirk 2001). Similarly, the native *Hymenoscyphus albidus* populations were displaced by an aggressive ash dieback agent (*H. fraxineus*) in Europe (e.g. Drenkhan et al. 2016).

A new wave of DED has occurred in Estonia since the 1990's, particularly in the areas grouped in this investigation as sample sites A and B. The losses of elms were suspected to be higher than during the previous era, i.e., the first half of the twentieth century (M. Hanso, pers. comm.). Likewise, in many other parts of Europe, and in some parts of Asia (Kirisits 2013) and North America (Dobbs et al. 2017) DED killed the majority of mature elm trees. The scarcity of elms (Kukk and Kull 2005) may be one reason for the slower spread of the pathogen, and we have only a few pure stands in limited or concentrated areas, e.g. in central Estonia (Kaar 2011). Another reason why some of the regions are unaffected by DED, or the degradation is not severe, can be explained by the scarcity of DED insect vectors (Voolma et al. 2000; Süda 2006) and also unattractiveness of *U. laevis* to them (Sacchetti et al. 1990; Santini and Faccoli 2013; Martín et al. 2018). This was observed also in this work at sites A and B, where elm bark beetles were found only on sampled trees of *U. glabra* and not on *U. laevis*. However, it is probable that invasive pathogen *O. novo-ulmi* subsp. *americana* may have been imported to the port city of Tallinn by infected plants, because there is no evidence that the pathogen was detected at any other location in Estonia (Table 3).

DED and health condition of elms

Both subspecies that cause DED are equally dangerous to elms regardless of the location, e.g., at urban sites or at rural locations; the difference was statistically insignificant ($p > 0.05$).

The assessment of elms during two non-consecutive years, 2014 and 2016, demonstrates the health status of trees at sub-sites A1 and B1, but the health conditions of elms cannot automatically be extrapolated to all of Estonia. This study demonstrates a disastrous decline of elm trees vitality, at particular sampling sites in Estonia, where the trees were affected by *O. novo-ulmi* subspecies, since about 22% of trees were dead after 2 years (see Fig. 5). It shows the same tendency as seen in the history of severe attacks of DED in other countries in Europe and North America (Phillips and Burdekin 1992; Schmidt 2006).

From 2014 to 2016, in Tallinn (sub-site A1) the elms died much quicker than in sub-site B1, located in the south-west of Estonia; ca. 25% of assessed trees were already dead after 24 months. A statistically significant ($p < 0.00001$) difference of elm health conditions between sub-sites A1 and B1 was demonstrated, apparently due to the different subspecies of the pathogen. Samples from sub-site A1 showed the presence of non-hybrid *O. novo-ulmi* subsp. *americana* by genes *col1* and *cu* (see Table 3), which is recognized as more aggressive to elms (Brasier and Kirk 2001) than non-hybrid *O. novo-ulmi* subsp. *novo-ulmi,* found at sub-site B1. Since another inventory at site B (Tihemetsa park in south-west Estonia) showed that 52% of assessed *U. glabra* trees died in 10 years (Laas and Treumuth 2006; Rist 2015), it may indicate that the *O. novo-ulmi* subsp. *novo-ulmi* that exists in site B is a less aggressive

pathogen (see Table 3). Nevertheless, population genetics at the genome level may give more information origin of the pathogens and pathogenicity.

In those regions where the occurrence of both subspecies of the pathogen overlap, their hybrids have also been found (Konrad et al. 2002; Dvořák et al. 2007) and even complex hybrids can occur (Brasier et al. 2004). Closest to Estonia, records of both subspecies come from the southern part of Norway and Sweden (Brasier and Kirk 2010). No hybrids were detected during this research. This can be explained by quite long distances between the sites (over 100 km) where different subspecies were found.

No other serious pathogens were isolated from symptomatic shoots from the elms at different sampling sites in Estonia (see Appendix Table 6); most of them are known endophytes (Blumenstein 2015; Martín et al. 2013b). It indicates that the most important pathogens on elms are subspecies of *O. novo-ulmi*. It is also important that in the northern Baltics the elms grow near the northern limit of their natural distribution area (Laasimer 1965), which increases their sensitivity to climate change and susceptibility to pathogens (Hanso and Drenkhan 2007, 2013). However, elms are native to Estonia and at the sampling sites only mature trees of Estonian origin were assessed (S. Järve, dendrologist, pers. comm.). Thus, we consider that hosts are similarly diverse as in native populations, but the elm populations are not genetically analysed in Estonia. The worst health situation for elms was in the Tallinn area (sub-site A1), not in other similar conditions in the northern and western parts of Estonia, and also at the sampling site D in eastern Estonia. The elms had been monitored by pathologists, arborists and other specialists for a longer period across Estonia. Furthermore, the differences in weather characteristics between sub-sites A1 and B1 were statistically insignificant (see Table 2). Additionally, differences in DED symptoms on elms were statistically insignificant ($p > 0.05$) in the urban space sites versus rural sites, incl. forests. It demonstrates that environmental characteristics (weather, site type, etc.) are not the causes of the devastating health conditions of elms in sub-site A1, particularly in Tallinn (see Fig. 1).

Only climate data (temperature and precipitation) were analysed to compare different sites. Other abiotic characteristics were not analysed in this work as those are unlikely diminish vitality of elms, because they are extremely hardy against abiotic stresses (Townsend and Douglass 2004; Scheffer et al. 2008; Büchel et al. 2016), adapt to city conditions (Santini et al. 2010) and lack specific soil type requirements (Buiteveld et al. 2014). The soils of comparable sites are sandy type (Umbri-Densic and Haplic Podzol to Albeluvisols) according to Astover et al. (2012) and the precise Estonian Soil Map (2018). However, with this kind of field experiment, the geographical difference of locations in sub-sites A1 and B1 was not ideal compared to a classical field trial, but it is still necessary to assess and analyse pathogen impact on natural populations, because it is not always possible to conduct classical field trials.

DED affects host species differently

Ulmus spp. have inherent differences in tolerance to DED (Guries and Smalley 2000; Townsend 2000; Venturas et al. 2014; Solla et al. 2014). The variation in susceptibility may depend on their anatomy and physiology, such as differences in vessels in the early wood, pit openings, other xylem features and branch sizes (Solla and Gil 2002; Martín et al. 2009, 2013a). *Ulmus glabra* was more affected by DED pathogens at the assessed sampling sites in Estonia (Fig. 3). *Ulmus laevis* was less affected ($p < 0.001$), similar to its characteristic in Poland (Łakomy et al. 2016) as well as results of inoculation tests in France (Pinon et al. 2005).

Some studies demonstrate that *U. laevis* is less attractive to the *Scolytus* beetles (Sacchetti et al. 1990; Santini and Faccoli 2013). This study confirms that finding (sites A and B), where elm bark beetles were found only on sampled trees of *U. glabra* and not on *U. laevis*. However, inoculation tests with *U. laevis* had also demonstrated

some susceptibility of this elm species (Pinon et al. 2005; Solla et al. 2005). Some elm cultivars usually stayed uninfected by DED pathogens, as was shown in a previous research work in Estonia (Aaspõllu 1999), but mature *U. glabra* 'Camperdownii' died due to DED in Tihemetsa park (sub-site B1). Still, some native mature elm trees seemed less susceptible to DED pathogens in heavily diseased areas in Estonia, suggesting that native elms species do not die out. These are viewed to be potential future trees for the environmental conditions of Estonia.

Alternatively, it has been suggested that resistant elm cultivars or hybrids (e.g., *Ulmus davidiana* var. *japonica* × *U. pumila* 'New Horizon') could be used in urban spaces (Brunet et al. 2013; Buiteveld et al. 2014). Nevertheless, since most of these elm hybrids have *U. pumila* as one of the parental species (Brunet et al. 2013), these hybrids do not grow well in northern climates, because *U. pumila* shoots are sensitive to late frosts in early spring, e.g., in Tallinn Botanical Garden, northern Estonia (A. Kaur, dendrologist, pers. comm.). In this study, all the assessed hybrid elms (20 trees) demonstrated some frost dieback symptoms on previous-year shoots. It clearly means that non-native species should be tested before large-scale planting in new conditions, such as north-eastern Europe.

DED pathogen identification

The pathogen cultures isolated from 76 different elms shoot samples showing typical symptoms of DED infection. Species detection of the causative agents of DED, *Ophiostoma novo-ulmi* and its subspecies, was carried out from isolated cultures by species-specific PCR primers (e.g. Gibb and Hausner 2005; Konrad et al. 2002). The primers were found to be effective only for pure cultures and were not species-specific when testing biological samples, e.g. symptomatic host tissues. Species-specific PCR primers are needed to quickly and reliably detect *Ophiostoma* species and subspecies from symptomatic host tissues. These primers will be useful for imported and exported planting material and for the general molecular monitoring of DED agents.

Conclusions

This study provides new information on Dutch elm disease in the north-eastern part of Europe, where the invasive pathogen *Ophiostoma novo-ulmi,* with its two subspecies (subsp. *novo-ulmi* and subsp. *americana*), was identified for the first time in Estonia. All 25 isolates, tested by *col1* and *cu* genes, were not hybrids. In the natural conditions of two non-consecutive calendar years at different sampling sites in the northern Baltics, the mean probability of native elm trees to die was ca. 22% by DED agents within 24 months. *Ophiostoma novo-ulmi* subsp. *americana* demonstrated higher aggressiveness toward elm trees in spite of the limitations inherent to the study; its victims died more than two times faster than elms infected by *O. novo-ulmi* subsp. *novo-ulmi*.

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Compliance with ethical standards The authors declare that ethical standards have been followed and that no human participants or animals were involved in this research.

Conflict of interest The authors declare that they have no competing interests.

Appendix

Table 6 List of other isolated and determined fungi from different elm trees' shoots in all sampling sites

No	Sampling				Host species	Molecular identification
	site	date	location	habitat		
1	A	02.07.2015	urban	park	*Ulmus glabra*	*Sphaeropsis ulmicola*
2	A1	14.07.2016	urban	street	*U. minor*	*Aureobasidium* sp.
3	A	31.07.2016	rural	road	*U. glabra*	*Phomopsis* sp.
4	A	31.07.2016	urban	park	*U. glabra*	*Dothiorella sarmentorum*
5	B	01.07.2015	urban	forest park	*U. glabra*	*Phomopsis* sp.
6	B	01.07.2015	urban	forest park	*U. glabra*	*Fusarium lateritum*
7	B	04.07.2015	urban	street	*U. glabra*	*Phomopsis* sp.
8	B	17.07.2015	urban	street	*U. glabra*	*Aureobasidium pullulans*
9	B	19.07.2015	rural	park	*U. glabra*	*Aureobasidium pullulans*
10	B	21.08.2015	rural	park	*U. glabra 'Camperdownii'*	*Cladosporium allicinum*
11	B	01.10.2015	rural	park	*U. glabra*	*Undosporium allicinium*
12	B	09.07.2016	urban	park	*U. glabra*	*Pleospolares* sp.
13	B1	10.07.2016	rural	park	*U. glabra*	*Phaeobotryon* sp.
14	B1	10.07.2016	rural	park	*U. glabra*	*Fusarium* sp.
15	B1	10.07.2016	rural	park	*U. glabra*	*Cladosporium* sp.
16	B1	10.07.2016	rural	park	*U. glabra*	*Phomopsis* sp.
17	B1	10.07.2016	rural	park	*U. glabra*	*Lophiostoma* sp.
18	B	20.07.2016	rural	forest	*U. glabra*	*Diaporthe* sp.
19	B	31.07.2016	rural	park	*U. glabra*	*Phomopsis* sp.
20	C	17.06.2016	urban	park	*U. glabra*	*Dothiorella ulmicola*
21	C	17.06.2016	urban	park	*U. glabra*	*Phoma* sp.
22	C	17.06.2016	urban	park	*U. glabra*	*Etypa crustata*
23	C	17.06.2016	urban	park	*U. glabra*	*Lophiostoma* sp.
24	C	17.06.2016	urban	park	*U. glabra*	*Boeremia exiqua* var. *exiqua*
25	C	17.06.2016	urban	park	*U. glabra*	*Cladosporium* sp.
26	C	30.06.2015	urban	street	*U. glabra*	*Sphaeropsis ulmicola*
27	C	30.06.2015	urban	street	*U. glabra*	*Mucor hiemalis f*
28	C	30.06.2015	urban	street	*U. glabra*	*Sphaeropsis ulmicola*
29	C	13.07.2016	urban	street	*U. hybrid*[a]	*Cladosporium* sp.
30	C	13.07.2016	urban	street	*U. hybrid*[a]	*Alternaria* sp.
31	C	13.07.2016	urban	street	*U. hybrid*[a]	*Leptosphaeria rubefaciens*
32	D	18.07.2016	rural	forest	*U. glabra*	*Mortierella hyalina*

[a] *Ulmus davidiana* var. *japonica* × *U. pumila* 'New Horizon'

References

Aaspõllu, A. (1999). Lehtpuude kultivarid Eestis (Cultivars of deciduous trees in Estonia) [in Estonian], H. Sander (ed.) *Dendroloogilised uurimused Eestis (*Dendrological researches in Estonia) (pp. 110-137).

Abner, O., Konsa, S., Lootus, K., & Sinijärv, U. (2007). *Eesti pargid 1* (Estonian parks 1) [in Estonian]. Keskkonnaministeerium, Muinsuskaitseamet, Varrak, Tallinn. 423 p.

Abner, O., Konsa, S., Lootus, K., & Sinijärv, U. (2012). *Eesti pargid 2* (Estonian parks 2) [in Estonian]. Keskkonnaministeerium, Muinsuskaitseamet, Varrak, Tallinn. 647 p.

Adamson, K., Drenkhan, R., & Hanso, M. (2015a). Invasive brown spot needle blight caused by *Lecanosticta acicola* in Estonia. *Scandinavian Journal of Forest Research., 30*(7), 587–593. https://doi.org/10.1080/02827581.2015.1041550.

Adamson, K., Kalvina, D., Drenkhan, R., Gaitnieks, T., & Hanso, M. (2015b). *Diplodia sapinea* is colonizing the native Scots pine (*Pinus sylvestris*) in the northern Baltics. *European Journal of Plant Pathology., 143*(2), 343–350. https://doi.org/10.1007/s10658-015-0686-8.

Adamson K., Laas M., Drenkhan R., & Hanso M. (2018). Quarantine pathogen Lecanosticta acicola, observed at its jump from an exotic host to the native Scots pine in Estonia. *Baltic Forestry, 24*(1), 36–41.

Adamson, K., Mullett, M. S., Solheim, H., Barnes, I., Müller, M. M., Hantula, J., et al. (2018b). Looking for relationships between the populations of *Dothistroma septosporum* in northern Europe and Asia. *Fungal Genetics and Biology, 110*, 15–25. https://doi.org/10.1016/j.fgb.2017.12.001.

Astover, A. Kõlli, R., Roostalu, H. Reintam, L., & Leedu, E. (2012). *Mullateadus* (Soil Science) [in Estonian]. Estonian University of Life Sciences. 486 p.

Atkinson, T. H. (2017). Scolytus. Bark and Ambrosia Beetles. Available online at http://www.barkbeetles.info/beetle_pix_tax_index.php#scolytus . Accessed 15 Dec 2017.

Barstow, M., & Harvey-Brown, Y. (2017). *Ulmus laevis*. The IUCN Red list of threatened species 2017: e.T61967009A61967013. Available online at https://doi.org/10.2305/IUCN.UK.2017-3.RLTS.T61967009A61967013.en, last accessed Dec. 12, 2018.

Barstow, M., & Rivers, M.C. (2017). *Ulmus glabra*. The IUCN Red list of threatened species 2017: e.T61966807A61966819. Available online at https://doi.org/10.2305/IUCN.UK.2017-3.RLTS.T61966807A61966819.en, last accessed Dec. 12, 2018.

Barstow, M., Rivers, M. C., & Harvey-Brown, Y. (2017). *Ulmus minor*. The IUCN Red list of threatened species 2017: e.T19218731A69047375. Available online at https://doi.org/10.2305/IUCN.UK.2017-3.RLTS.T19218731A69047375.en, last accessed Dec. 12, 2018.

Bernier, L., Aoun, M., Bouvet, G. F., Comeau, A., Dufour, J., et al. (2014). Genomics of the Dutch elm disease pathosystem: Are we there yet? *iForest Biogeosciences and Forestry Collection: 3rd International Elm Conference* "*The elms after 100 years of Dutch Elm disease*"*, 8*, 149-157. (A. Santini, et al., ed.) Florence, Italy. https://doi.org/10.3832/ifor1211-008.

Blumenstein, K. (2015). *Endophytic Fungi in Elms*. PhD thesis, SLU. 84 p.

Brasier, C. M. (2008). The biosecurity threat to the UK and global environment from international trade in plants. *Plant Pathology, 57*, 792–808. https://doi.org/10.1111/j.1365-3059.2008.01886.x.

Brasier, C. M., & Buck, K. W. (2001) Rapid evolutionary changes in a globally invading fungal pathogen (Dutch elm disease). *Biological Invasions, 3*, 223–233.

Brasier, C. M., & Kirk, S. A. (2001). Designation of the EAN and NAN races of *Ophiostoma novo-ulmi* as subspecies. *Mycological Research, 105*(5), 547–554. https://doi.org/10.1017/S0953756201004087.

Brasier, C. M., & Kirk, S. A. (2010). Rapid emergence of hybrids between the two subspecies of *Ophiostoma novo-ulmi* with a high level of pathogenic fitness. *Plant Pathology, 59*, 186–199. https://doi.org/10.1111/j.1365-3059.2009.02157.x.

Brasier, C., Buck, K., Paoletti, M., & Crawford, L. (2004). Molecular analysis of evolutionary changes in populations of *Ophiostma novo-ulmi*. *Investigación agraria*. *Sistemas y recursos forestales, 13*(1), 93–103. https://doi.org/10.5424/817.

Brunet, J., Zalapa, J., Pecori, F., & Santini, A. (2013). Hybridization and introgression between the exotic Siberian elm, *Ulmus pumila*, and the native field elm, *U. minor*, in Italy. *Biological Invasions, 15*, 2717–2730. https://doi.org/10.1007/s10530-013-0486-z.

Büchel, K., Fenning, T., Gershenzon, J., Hilker, M., & Meiners, T. (2016). Elm defence against herbivores and pathogens: Morphological, chemical and molecular regulation aspects. *Phytochemistry Reviews, 15*, 961–983. https://doi.org/10.1007/s11101-015-9442-0.

Buiteveld, J., Van Der Werf, B., & Hiemstra, J. A. (2014). Comparison of commercial elm cultivars and promising unreleased Dutch clones for resistance to *Ophiostoma novo-ulmi*. *iForest Biogeosciences and Forestry Collection: 3rd International Elm Conference* "*The elms after 100 years of Dutch Elm disease*"*, 8*, 158-164. (A. Santini, et al., ed.) Florence, Italy. https://doi.org/10.3832/ifor1209-008.

Caulton, E., Aitken, W., & Rashid, N. (1998). Aerobiological aspects of elm (*Ulmus* spp.) in south-East Scotland in relation to elm decline from Dutch Elm disease (1976-1996). *Aerobiologia, 14*, 147–153.

Collin, E. (2002). Strategies and guidelines for the conservation of the genetic resources of *Ulmus* spp. *"Noble Hardwoods" network*. In S. Borelli (Ed.), *Report of the fourth and fifth meetings, Sept. 1999 and May 2001* (pp. 50–67). Rome: International Plant Genetic Resources Institute.

Corfixen, P., & Parmasto, E. (2005). *Hymenochaete ulmicola* sp. nov. (Hymenochaetales). *Mycotaxon, 91*, 465–469.

Dehnen-Schmutz, K., Holdenrieder, O., Jeger, M. J., & Pautasso, M. (2010). Structural change in the international horticultural industry: Some implications for plant health. *Scientia Horticulturae, 125*, 1–15. https://doi.org/10.1016/j.scienta.2010.02.017.

Dobbs, C., Martinez-Harms, M. J., & Kendal, D. (2017). The ecosystem service concept and its importance for socio-ecological systems. In *Routledge handbook of urban forestry* (pp. 51–64). London: Routledge.

Drenkhan, R., Sander, H., & Hanso, M. (2014). Introduction of Mandshurian ash (*Fraxinus mandshurica* Rupr.) to Estonia:

Is it related to the current epidemic on European ash (*F. excelsior* L.)? *European Journal of Forest Research, 133*(5), 769–781. https://doi.org/10.1007/s10342-014-0811-9.

Drenkhan, R., Adamson, K., & Hanso, M. (2015). Fraxinus sogdiana, a Central Asian ash species, is susceptible to Hymenoscyphus fraxineus . *Plant Protection Science, 51*(No. 3), 150–152.

Drenkhan, R., Riit, T., Adamson, K., & Hanso, M. (2016). The earliest samples of *Hymenoscyphus albidus* vs. *H. fraxineus* in Estonian mycological herbaria. *Mycological Progress, 15*, 835–844. https://doi.org/10.1007/s11557-016-1209-5.

Drenkhan, R., Solheim, H., Bogacheva, A., Riit, T., Adamson, K., Drenkhan, T., Maaten, T., & Hietala, A. M. (2017). *Hymenoscyphus fraxineus* is a leaf pathogen of local *Fraxinus* species in the Russian Far East. *Plant Pathology, 66*, 490–500. https://doi.org/10.1111/ppa.12588.

Dvořák, M., Tomšovský, M., Jankovský, L., & Novotný, D. (2007). Contribution to Identify the Causal Agents of Dutch Elm Disease in the Czech Republic. *Plant Protection Science, 43*(4), 142–145.

EPPO (2017). EPPO Global database. Available online at https://gd.eppo.int.; last accessed Dec. 15, 2017.

Estonian Soil Map (2018). Estonian land board geoportal. Available online at https://geoportaal.maaamet.ee/eng/Maps-and-Data/Estonian-Soil-Map-p316.html, last accessed Dec. 31, 2018.

Flower, C. E., Slavicek, J. M., Lesser, D., Eshita, S., & Pinchot, C. C. (2017). Canopy decline assessments in American elm after inoculation with different doses of *Ophiostoma ulmi* and *O. novo-ulmi*. In: *Proceedings of the American Elm Restoration Workshop 2016.* C. C. Pinchot, K. S. Knight, L. M. Haugen, C. E. Flower, & J. M. Slavicek (eds.). United States Department of Agriculture (pp. 24-29). https://doi.org/10.2737/NRS-GTR-P-174.

Gardes, M., & Bruns, T. D. (1993). ITS primers with enhanced specificity for basidiomycetes – Application of the identification of mycorrhizae and rusts. *Molecular Ecology, 2*, 113–118. https://doi.org/10.1111/j.1365-294X.1993.tb00005.x.

Ghelardini, L., Luchi, N., Pecori, F., Pepori, A. L., Danti, R., Rocca, G. D., et al. (2017). Ecology of invasive forest pathogens. *Biological Invasions, 19*, 3183–3200. https://doi.org/10.1007/s10530-017-1487-0.

Gibbs, J. N. (1978) Intercontinental Epidemiology of Dutch Elm Disease. *Annual Review of Phytopathology, 16*(1), 287–307.

Gibb, E. A., & Hausner, G. (2005). Optional mitochondrial introns and evidence for a homing-endonuclease gene in the mtDNA rnl gene in *Ophiostoma ulmi* s. lat. *Mycological Research, 109*(10), 1112–1126. https://doi.org/10.1017/S095375620500376X.

Guries, R. P., & Smalley, E. B. (2000). Once and future elms: Classical and molecular approaches to Dutch elm disease resistance. In C. P. Dunn (Ed.), *The Elms* (pp. 231–248). Boston: Springer.

Hall, T. A. (2013). Biological sequence alignment editor and analysis program for Windows 95/98/NT/2000/XP/7. *BioEdit*. Available online at http://www.mbio.ncsu.edu/bioedit/bioedit.html; last accessed Dec. 15, 2017.

Hannunen, S., & Marinova-Todorova, M. (2016). Pest risk assessment for Dutch elm disease. *Evira*. Available online at https://www.evira.fi/globalassets/tietoa-evirasta/julkaisut/julkaisusarjat/kasvit/pest-risk-assessment-for-dutch-elm-disease.pdf; last accessed Dec. 15, 2017.

Hanso, M., & Drenkhan, R. (2007). Metsa- ja linnapuud ilmastiku äärmuste vaevas (Trees in forests and towns are suffering from the extreme weather conditions) [in Estonian]. *Eesti Loodus (Estonian Nature), 58*(4), 6–13.

Hanso, M., & Drenkhan, R. (2009). *Diplodia pinea* is a new pathogen on Austrian pine (*Pinus nigra*) in Estonia. *Plant Pathology, 58*(4), 797–797. https://doi.org/10.1111/j.1365-3059.2009.02082.x.

Hanso, M., & Drenkhan, R. (2013). Simple visualization of climate change for improving the public perception in forest pathology. *Forestry Studies, 58*, 37–45. https://doi.org/10.2478/fsmu-2013-0004.

Hillier N (1991). *The Hillier Manual of Trees & Shrubs*. J. G. Hillier, & A. J. Coombes (eds.), (pp. 508–512). Winchester: David & Charles.

IUCN (2018). The IUCN Red list of threatened species. Version 2018-2. Available online at: www.iucnredlist.org, last accessed Dec.12, 2018.

Jüriado, I., Liira, J., & Paal, J. (2009). Diversity of epiphytic lichens in boreo-nemoral forests on the north-Estonian limestone escarpment: The effect of tree level factors and local environmental conditions. *The Lichenologist, 41*(1), 81–96. https://doi.org/10.1017/S0024282909007889.

Kaar, E. (2011). Jalakas ja künnapuu Eestis (elms in Estonia) [in Estonian]. *Eesti Loodus (Estonian Nature)., 62*(3).

Kalamees, K. (2011). Roosa võrkheinik: Seenharuldus jalakal (wrinkled peach: Rare fungus on elms) [in Estonian]. *Eesti Loodus (Estonian Nature), 62*(10), 41–41.

Kalda, A. (1995). Broadleaved forests in Estonia. *Consortium Masingii.* Aaviksoo, K, Kull, K., Paal, J., & Trass, H. (eds.) (pp.89-95).

Keskkonnaagentuur (2015). *Eesti meteoroloogia aastaraamat 2014 (Estonian Meteorological Yearbook 2014)* [In Estonian]. A. Kallis, K. Loodla, E. Tillmann, M. Krabbi, R. Pärg, K. Vint, A. Jõeveer, & E. Juust (compilers). Eesti Meteoroloogia ja Hüdroloogia Instituut. Available online at http://www.ilmateenistus.ee/wp-content/uploads/2016/02/aastaraamat_2014.pdf, last accessed Dec. 15, 2017.

Keskkonnaagentuur (2016). *Eesti meteoroloogia aastaraamat 2015 (Estonian Meteorological Yearbook 2015* [In Estonian]. A. Kallis, K. Loodla, E. Tillmann, M. Krabbi, R. Pärg, K. Vint, & A. Jõeveer (compilers). Eesti Meteoroloogia ja Hüdroloogia Instituut. Available online at http://www.ilmateenistus.ee/wp-content/uploads/2016/03/aastaraamat_2015.pdf, last accessed Dec. 15, 2017.

Keskkonnaagentuur (2017). *Eesti meteoroloogia aastaraamat 2016 (Estonian Meteorological Yearbook 2016)* [In Estonian]. K. Loodla, E. Tillmann, A. Kallis, R. Pärg, K. Vint, E. Juust, & M. Krabbi (compilers). Eesti Meteoroloogia ja Hüdroloogia Instituut. Available online at http://www.ilmateenistus.ee/wp-content/uploads/2013/01/aastaraamat_2016.pdf, last accessed Dec. 15, 2017.

Kirisits, T. (2013). Dutch elm disease and other *Ophiostoma* diseases. In P. Gonthier & G. Nicolotti (Eds.), *Infectious forest diseases* (pp. 256–282). Wallingford: CABI Publishing.

Konrad, H., Kirisits, T., Riegler, M., Halmschla, E., & Stauffer, C. (2002). Genetic evidence for natural hybridization between the Dutch elm disease pathogens *Ophiostoma novo-ulmi* ssp.

novo-ulmi and *O. novo-ulmi* ssp. *americana*. *Plant Pathology, 51*, 78–84. https://doi.org/10.1046/j.0032-0862.2001.00653.x.

Kristian, J. (1939). Jalakapuistu (Elmstand) [in Estonian]. *Estonian Forest (Eesti mets), 5*, 171–172.

Kukk, T., & Kull, T. (2005). *Eesti taimede levikuatlas (Atlas of the Estonian Flora) [in Estonian]* (p. 528). Tartu: Eesti Maaülikooli Põllumajandus- ja Keskkonnainstituut.

La Porta, N., Capretti, P., Thomsen, I. M., Kasanen, R., Hietala, A. M., & Von Weissenberg, K. (2008). Forest pathogens with higher damage potential. In Symposium contribution: The Effects of Climate Change on Tree Diseases. *Canadian Journal of Plant Pathology, 30*, 177–195. https://doi.org/10.1080/07060661.2008.10540534.

Laas, E., & Treumuth, S. (2006). *Voltveti mõisapargi ja Tihemetsa arboreetumi dendroloogiline inventuur* (Dendrological inventory of Voltvet manor park and Tihemetsa arboretum) [in Estonian]. Tartu, 78 p.

Laasimer, L. (1965). *Eesti NSV taimkate (Vegetation of Estonian SSR) [in Estonian]* (p. 421). Tallinn: Valgus.

Łakomy, P., Kwaśna, H., Kuźmiński, R., Napierała-Filipiak, A., Filipiak, M., Behnke, K., & Behnke-Borowczyk, J. (2016). Investigation of *Ophiostoma* population infected elms in Poland. *Dendrobiology, 76*, 137–144. https://doi.org/10.12657/denbio.076.013.

Leht, M. (2018). *Ulmus laevis*. eBiodiversity, available online at http://vana.elurikkus.ut.ee/kirjeldus.php?lang=eng&id=8066, last accessed Dec. 12, 2018.

Lepik, E. (1940). Estonia: The elm disease in the country. *International Bulletin of Plant Protection, International Review of Agriculture., 31*, 2–2.

Liebhold, A. M., Brockerhoff, E. G., Kalisz, S., Nuñez, M. A., Wardle, D. A., & Wingfield, M. J. (2017). Biological invasions in forest ecosystems. *Biological Invasions, 19*, 3437–3458. https://doi.org/10.1007/s10530-017-1458-5.

Lilleleht, V. (Ed.). (2008). *Eesti Punane Raamat (Red data book of Estonia) [in Estonian]*. Tartu: Commission for Nature Conservation of the Estonian Academy of Sciences.

Martín, J. A., Solla, A., Esteban, L. G., de Palacios, P., & Gil, L. (2009). Bordered pit and ray morphology involvement in elm resistance to *Ophiostoma novo-ulmi*. *NRC Research Press* (pp. 420-429). https://doi.org/10.1139/X08-183.

Martín, J. A., Fuentes-Utrilla, P., Gil, L., & Witzell, J. (2010). Broadleaved forests in southern Sweden: Management for multiple goals. *Ecological Bulletins, 53*, 209–224.

Martín, J. A., Solla, A., Ruiz-Villar, M., & Gil, L. (2013a). Vessel length and conductivity of *Ulmus* branches: Ontogenetic changes and relation to resistance to Dutch elm disease. *Trees, 27*, 1239–1248. https://doi.org/10.1007/s00468-013-0872-2.

Martín, J. A., Witzell, J., Blumenstein, K., Rozpedowska, E., Helander, M., Sieber, T. N., & Gil, L. (2013b). Resistance to Dutch Elm disease reduces presence of xylem endophytic fungi in elms (*Ulmus* spp.). *PLoS ONE, 8*(2), e56987. https://doi.org/10.1371/journal.pone.0056987.

Martín, J. A., Sobrino-Plata, J., Rodríguez-Calcerrada, J., Collada, C., & Gil, L. (2018). Breeding and scientific advances in the fight against Dutch elm disease: Will they allow the use of elms in forest restoration? *New Forests*. https://doi.org/10.1007/s11056-018-9640-x.

Menkis, A., Östbrant, I.-L., Wågström, K., & Vasaitis, R. (2016). Dutch elm disease on the island of Gotland: Monitoring disease vector and combat measures. *Scandinavian Journal of Forest Research, 31*(3), 237–241. https://doi.org/10.1080/02827581.2015.107688.

Motiejūnaitė, J., Kutorga, E., Kasparavičius, J., Lygis, V., & Norkutė, G. (2016). New records from Lithuania of fungi alien to Europe. *Mycotaxon, 131*, 49–60. https://doi.org/10.5248/131.49.

Müller, M. M., Hamberg, L., & Hantula, J. (2016). The susceptibility of European tree species to invasive Asian pathogens: A literature based analysis. *Biological Invasions, 18*, 2841–2851. https://doi.org/10.1007/s10530-016-1174-6.

Paal, J. (1998). Rare and threatened plant communities of Estonia. *Biodiversity and Conservation, 7*, 1027–1049. https://doi.org/10.1023/A:1008857014648.

Phillips, D. H., & Burdekin, D. A. (1992). *Diseases of forest and ornamental trees* (pp. 299–314). London, Basingstoke: Palgrave Macmillan.

Pinon, J., Husson, C., & Collin, E. (2005). Susceptibility of native French elm clones to *Ophiostoma novo-ulmi*. *Annals of Forest Science, 62*, 689–696. https://doi.org/10.1051/forest:2005066.

Pipe, N. D., Buck, K. W., & Brasier, C. M. (1997). Comparison of the cerato-ulmin (cu) gene sequences of the Himalayan Dutch elm disease fungus *Ophiostoma himal-ulmi* with those of *O. ulmi* and *O. novo-ulmi* suggests that the cu gene of *O. novo-ulmi* is unlikely to have been acquired recently from *O. himal-ulmi*. *Mycological Research, 101*(4), 415–421.

Pitney Bowes Software. (2015). MapInfo Pro Version 15.0.

Raudsaar, M., Pärt, E., & Adermann, V. (2016). Volume of tree species in forest land. In *Yearbook Forest 2014*, 5, Keskkonnaagentuur (ed.) (p. 20).

Rist, E. (2015). *Tihemetsa pargi inventuur (Inventory of Tihemetsa park) [in Estonian]*. Tihemetsa: Lõputöö.

Rosenvald, R., Drenkhan, R., Riit, T., & Lõhmus, A. (2015). Towards silvicultural mitigation of the European ash (*Fraxinus excelsior*) dieback: The importance of acclimated trees in retention forestry. *Canadian Journal of Forest Research, 45*(9), 1206–1214. https://doi.org/10.1139/cjfr-2014-0512.

Rytkönen, A., Lilja, A., Petäistö, R.-L., & Hantula, J. (2008). Irrigation water and stem lesions on *Betula pendula* in a forest nursery. *Scandinavian Journal of Forest Research, 23*, 404–411. https://doi.org/10.1080/02827580802419034.

Rytkönen, A., Lilja, A., Drenkhan, R., Gaitnieks, T., & Hantula, J. (2011). First record of *Chalara fraxinea* in Finland and genetic variation among isolates sampled from Åland, mainland Finland, Estonia and Latvia. *Forest Patholology, 41*, 169–174. https://doi.org/10.1111/j.1439-0329.2010.00647.x.

Saarse, L., & Veski, S. (2001). Spread of broad-leaved trees in Estonia. *Proceedings of the Estonian Academy of Sciences, Geology, 50*(1), 51–65.

Sacchetti, P., Tiberi, R., & Mittempergher, L. (1990). Preference of *Scolytus multistriatus* Marsham in twig-crotch feeding on two elm species. *Redia, 73*(2), 347–354.

Santini, A., & Faccoli, M. (2013). Dutch elm disease and elm bark beetles: A century of association. *iForest Biogeosciences and Forestry Collection: 3rd International Elm Conference* "*The elms after 100 years of Dutch Elm disease*"*, 8*, 126-134. (A.

Santini, et al., ed.) Florence, Italy. https://doi.org/10.3832/ifor1231-008.

Santini, A., Pecori, F., Pepori, A. L., Ferrini, F., & Ghelardini, L. (2010). Genotype×environment interaction and growth stability of several elm clones resistant to Dutch elm disease. *Forest Ecology and Management, 260*, 1017–1025.

Scheffer, R. J., Voeten, J. W., & Guries, R. P. (2008). Biological control of Dutch Elm disease. *Plant Disease, 92*(2), 192–200. https://doi.org/10.1094/PDIS-92-2-0192.

Schmidt, O. (2006). *Wood and tree fungi. Biology, damage, protection and use* (pp. 168–170). Berlin, Heidelberg: Springer-Verlag.

Solheim, H., Eriksen, R., & Hietala, A. (2011). Dutch elm disease has currently a low incidence on wych elm in Norway. *Forest Pathology, 41*, 182–188. https://doi.org/10.1111/j.1439-0329.2010.00650.x.

Solla, A., & Gil, L. (2002). Xylem vessel diameter as a factor in resistance of *Ulmus minor* to *Ophiostoma novo-ulmi*. *Forest Pathology, 32*(2), 123–134. https://doi.org/10.1046/j.1439-0329.2002.00274.x.

Solla, A., Bohnens, J., Collin, E., Diamandis, S., Franke, A., Gil, L., et al. (2005). Screening European elms for resistance to *Ophiostoma novo-ulmi*. *Forest Science, 51*(2), 134–141. https://doi.org/10.1093/forestscience/51.2.134.

Solla, A., Dacasa, M. C., Nasmith, C., Hubbes, M., & Gil, L. (2008). Analysis of Spanish populations of *Ophiostoma ulmi* and *O. novo-ulmi* using phenotypic characteristics and RAPD markers. *Plant Pathology, 57*, 33–44. https://doi.org/10.1111/j.1365-3059.2007.01692.x.

Solla, A., López-Almansa, J., Martín, J., & Gil, L. (2014). Genetic variation and heritability estimates of *Ulmus minor* and *Ulmus pumila* hybrids for budburst, growth and tolerance to *Ophiostoma novo-ulmi*. *Biogeosciences and Forestry, 8*, 422–430. https://doi.org/10.3832/ifor1227-007.

Süda, I. (2006). Jalaka-maltsaürask (*Scolytus triarmatus* (Eggers, 1912)) – uus üraskiliik Baltikumis (*Scolytus triarmatus* (Eggers, 1912) – A new bark beetle in the Baltics) [in Estonian]. *Metsanduslikud uurimused (Forestry studies), 44*, 112–117.

Tamm, H. (2007). Taimestik ja taimkate (Vegetation and plantcover) [in Estonian]. *Eesti parkide almanahh (Almanac of Estonian Parks)* I. Muinsuskaitseamet, Keskkonnaministeerium (pp. 92-97).

Thor, G., Johansson, P., & Jonsson, M. T. (2010). Lichen diversity and red-listed lichen species relationships with tree species and diameter in wooded meadows. *Biodiversity and Conservation, 19*, 2307–2328. https://doi.org/10.1007/s10531-010-9843-8.

Townsend, A. M. (2000). USDA genetic research on Elms. In C. P. Dunn (Ed.), *The Elms* (pp. 271–278). Boston: Springer.

Townsend, A. M., & Douglass, L. W. (2004). Evaluation of elm clones for tolerance to Dutch Elm disease. *Journal of Arboriculture, 30*(3), 179–184.

Tziros, G. T., Nakopoulou, Z. G., Perlerou, C., & Diamandis, S. (2017) Current status of the Dutch elm disease pathogen populations affecting *Ulmus minor* in Greece. *Forest Pathology.* Available online at https://doi.org/10.1111/efp.12323, last accessed Dec. 31, 2018.

Venturas, M., Lopez, R., Martín, J. A., Gasco, A., & Gil, L. (2014). Heritability of *Ulmus minor* resistance to Dutch elm disease and its relationship to vessel size, but not to xylem vulnerability to drought. *Plant Pathology, 63*(3), 500–509.

Voolma, K., Õunap, H., & Süda, I. (2000). *Eesti putukate levikuatlas, 2: Ürasklased – Scolytidae (Distribution maps of Estonian insects, 2: Scolytidae) [in Estonian]* (p. 84). Tartu: Eesti Loodusfoto.

Voolma, K., Mandelshtam, M. J., Shcherbakov, A. N., Yakovlev, E. B., Õunap, H., Süda, I., et al. (2004). Distribution and spread of bark beetles (Coleoptera: Scolytidae) around the Gulf of Finland: A comparative study with notes on rare species of Estonia, Finland and North-Western Russia. *Entomologica Fennica, 15*(4), 198–210.

White, T. J., Bruns, T. D., Lee, S., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In M. A. Innis, D. H. Gelfand, J. J. Sninsky, & T. J. White (Eds.), *PCR protocols: A guide to methods and applications* (pp. 315–322). New York: Academic Press.

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The extensive damage to elms by Dutch elm disease agents and their hybrids in northwestern Russia

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ABSTRACT

Elms are important amenity trees in the European part of Russia, incl. the St. Petersburg and Leningrad Region. The native species *Ulmus glabra* and *U. laevis* mainly grow there, on the northern border of their natural range. Hybrid elm cultivars have been planted since the early 2000s. Thousands of elms suffer from Dutch elm disease in the Leningrad Region. We found that in the north-western part of Russia *Ophiostoma novo-ulmi* subsp. *novo-ulmi* and *O. novo-ulmi* subsp. *americana* as well as hybrids of these subspecies are aggressive agents of DED on elms. According to *in vitro* experiments of hybrids in pure cultures, *O. novo-ulmi* subsp. *americana* x *novo-ulmi* had the highest growth rate, and *O. novo-ulmi* subsp. *novo-ulmi* the lowest growth rate.

Ulmus glabra (>40 years old) was affected the most, with ca. 20 % of assessed trees found to already be dead and only 56 % found to be in good condition. The documented health among young elm cultivars (<25 years old) revealed that only 4% were dead, and 91 % were healthy or only experiencing some branch loss. *Ulmus laevis* (with 74 % of individuals healthy or with some branch loss, and 5% dead) was significantly healthier compared to *U. glabra*. In the city, the health of all assessed elm species was significantly better in greenspaces than next to highways.

As the potential vectors of DED, *Scolytus scolytus*, *S. multistriatus* and *S. pygmaeus* are registered in the assessed region.

1. Introduction

Warmer climate enables elms to grow in new northern locations (Drobyshev, 2001). Widening global trade increases the risk of invasion of new pests (Brasier, 2008; Hemery et al., 2010) and pathogens (Drenkhan et al., 2020; Santini et al., 2013), without any effective prevention to minimize the risk (Dickie et al., 2017; Hulme, 2009). In recent years several invasive alien pathogens have been found in northern Europe, e.g. *Diplodia sapinea, Dothistroma septosporum, Entoleuca mammata, Hymenoscyphus fraxineus, Lecanosticta acicola* (Adamson et al., 2015a,2015b 2018a, 2018b; Agan et al., 2020; Cleary et al., 2019; Drenkhan et al., 2015, 2016; Hanso and Drenkhan, 2009; Laas et al., 2019; Lutter et al., 2019; Müller et al., 2019; Mullett et al., 2018, 2021; Rosenvald et al., 2015).

The Leningrad Region is in the north-western part of Russia and has borders with EU countries, Finland and the Baltic states. Saint Petersburg, the capital of the region, was founded in 1703 (Ignatieva, 2005). St. Petersburg experiences a humid and cold climate with frequent floods, wind and storms, and the hydrological and geological peculiarities of the Neva delta. It is the second largest city in Russia with a total area of 1430 sq. km (St. Petersburg's Parks and Gardens, 2019), around 30 % of which is urban greenspace (Nilsson et al., 2007) with a unique urban planning structure, incl. parks and gardens, that has adjustments for local climatic conditions (Ignatieva, 2005). For more than three hundred years heritage landscape protection in St. Petersburg has preserved the authenticity of its components because of its special perspective (Ignatieva, 2005).

Elms are one of the main amenity tree species in Europe and also in the north-western part of Russia (Firsov and Bulgakov, 2017), and are the second most important trees in the green areas, e.g. parks, gardens and squares, allées and streets, in courtyards of apartment houses and enterprises of St. Petersburg because they grow well in urban

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environments (Ignatieva and Konechnaya, 2004; Trubacheva et al., 2014). Elms have been used in the greenspaces since 1703 (Ignatieva et al., 2011). Native elms for planting (*Ulmus laevis* Pall. and *U. glabra* Huds.) were purchased from the Baltic countries and local nurseries (Trubacheva et al., 2014) or have naturally regenerated in stands and parks, especially during the first half of the 20th century (Golovach, 1980). Most of the allées were created in the 19th century and re-constructed several times after 1945, and one of the main elm species planted was *Ulmus laevis* (Ignatieva et al., 2011). Also, in three different regions of the city 447 selected hybrid elms (cultivar and origin unknown) potentially resistant to DED were planted during the years 2006–2013 to keep elms continuously represented in the green areas of St. Petersburg (Shcherbakova and Mandelshtam, 2014).

The health status of elms in St. Petersburg has worsened substantially since 1995, starting from the southern part of the city, i.e. in the Pushkin district (Markova, 2000). By 2000, 185 trees had been found dead in the same area. Typical Dutch elm disease symptoms appeared some years later. Twenty-nine greenspaces (e.g. parks, allées) were recorded as afflicted by DED in St. Petersburg in 2002, 42 in 2006, 76 in 2007 and a total of 700 in 2015 (Dorofeeva and Tyupina, 2002; Selikhovkin et al., 2010).

In recent years, the death of elms has been catastrophic and now affects the entire territory of the city and the nearest suburbs (Dorofeeva, 2008; Selikhovkin et al., 2010; Shcherbakova and Mandelshtam, 2014). Since 2007, the Committee on Environmental Management, Environmental Protection and Ensuring Ecological Safety of St. Petersburg has regularly monitored the spread of DED in the city. By 2008, the total number of dead trees was at least 2500 due to the activity of bark beetles and DED (Fedorova, 2009). According to the data of the Committee for City Improvement and Roads of St. Petersburg, the damaged area of DED increased by 3.5 times during the period from 2009 to 2015. In 2015, more than 5000 dead elms were cut down.

The first record of DED appeared in the western regions of the USSR and south-west Asia in 1936 (Moschenikova and Vjaznikova, 2016), where the agent then probably was *O. ulmi* (Brasier and Buck, 2001). *Ophiostoma novo-ulmi* subsp. *novo-ulmi* and *O. novo-ulmi* subsp. *americana* now prevail in Europe (Brasier et al., 2004; Brasier and Buck, 2001; Martín et al., 2010). The closest occurrence of the *O. novo-ulmi* subspecies to the Leningrad Region was documented in Estonia in 2013 (Jürisoo et al., 2019). In the eastern part of Europe, hybrids between the two subspecies were, by that time, already detected in Hungary (Brasier et al., 2004), Poland (Brasier and Kirk, 2010), in the Czech Republic (Dvořák et al., 2007) in Lithuania (Motiejūnaitė et al., 2016) and Latvia (Matisone et al., 2020).

In neighboring Finland, DED caused by *O. ulmi* was reported in the middle of the last century (1952–1968) (Hintikka, 1974), but now DED agent is no longer present (EPPO Global Database, 2019; Hannunen and Marinova-Todorova, 2016). Despite these nearby occurrences, insect vectors (e.g. *Scolytus* spp.) capable of spreading DED agents have not been found in Finland (Hannunen and Marinova-Todorova, 2016; Hantula, 2021, Voolma et al., 2004), but several of them are present in Estonia (Voolma et al., 2000, 2004; Jürisoo et al., 2021). The northernmost findings of *Scolytus scolytus* Fabricius and *Scolytus multistriatus* Marsham have been found in St. Petersburg city parks (Voolma et al., 2004). *Scolytus pygmaeus* Fabricius as a new pest of elms was first found in 2012 in. St. Petersburg (Shcherbakova and Mandelshtam, 2014). Therefore, our investigation represents the north-easternmost survey of DED in Europe.

The aim of this research was to assess the health of elms in greenspaces of north-western Russia. The specific objectives were (1) to isolate, identify and physiologically characterize more precisely than previous reports the causative agents of DED in the north-western part of Russia, (2) to monitor the health status of elms and bark beetle occurrence in different sampling sites and habitats.

2. Material and methods

2.1. Study areas and survey of Ulmus spp

The survey was carried out visually by applying five crown vitality classes: 1 = healthy, 2 = up to 25 % branch loss, 3 = up to 50 % branch loss, 4 = dying, with less than 50 % live crown remaining, and 5 = dead with no live branches in 2016 (see Jürisoo et al., 2019).

Study areas were selected in parks, allées and greenspaces between multi-storey buildings in different regions of St. Petersburg, its southern suburb Pushkin (Tsarskoje Selo, plot 1) and in Vyborg, near the Finland border (plot 11), as well as along the highways from St. Petersburg to Vyborg and to Moscow (plot 10) (Fig. 1).

All sampling sites of elm trees were mapped, the species determined, and the crown conditions of trees assessed. Identification of *Ulmus* species was performed according to Hillier Nurseries (1991). Identity of hybrids requires DNA analysis, so we were only able to classify hybrid elms to genus (e.g. *Ulmus* spp.) (Table 1).

Elm trees with foliage symptoms such as wilting, yellowing and browning of leaves were regarded as affected by DED (Solheim et al., 2011), and samples for laboratory analyses were collected from those trees.

The maps were compiled using MapInfo Professional version 15 (Pitney Bowes Software, 2015).

2.2. Beetles on assessed trees

On assessed trees the bark beetle species composition was analyzed. Insect species were determined by B. G. Popovichev and M. J. Mandelshtam, based on gallery characteristics and the morphology of beetles. Only the lower part of the trunk was examined, up to the height of two meters. Maturation feeding of beetles in the canopy of elms has not been detected.

2.3. Climate

St. Peterburg's climate data (see Table 2) are summarized according to Bulygina et al. (2019).

3. Fungal isolation, DNA extraction, PCR and sequencing

Pathogens and other fungi (Appendix A) were isolated from the symptomatic shoots as in Jürisoo et al. (2019). Briefly, the bark of symptomatic shoots was peeled off with a sterile scalpel and a layer of wood was removed up to brown rings in the xylem. Then the small pieces of the infected wood tissue were placed on sterile MEA (Malt Extract Agar) and incubated at room temperature for 1–2 weeks. Then after several transfers to fresh MEA plates and cultivation, all isolates were transferred onto MEA plates covered with sterile cellophane membrane (British Cellophane Ltd., U.K.). After an incubation of ca. 1–2 weeks at room temperature, hyphal mass from culture edges was removed and stored at −20 °C until used for DNA isolation.

DNA extraction from isolates, PCR and sequencing were performed for the ITS region of the rRNA gene, col1 and cu genes (see Jürisoo et al., 2019). Species-specific PCR primers (SSPP) mtsr1 and mtsr2 (Gibb and Hausner, 2005) were used for quick detection of *O. ulmi* and *O. novo-ulmi* from mycelial DNA. *Ophiostoma* sp. and other fungi were detected using the ITS PCR primers ITS1-F (Gardes and Bruns, 1993) and ITS4 (White et al., 1990), carried out as described by Drenkhan et al. (2014). Amplified fragments of the two genes (*col1* and *cu*) were digested by restriction enzymes *Hph* I for gene *cu* and *Bfa* I for gene *col1* (New England Biolabs, USA), to distinguish the two *O. novo-ulmi* subspecies (Dvořák et al., 2007; Konrad et al., 2002; Tziros et al., 2017) according to the manufacturer's instructions of enzymes with some modifications as described by Jürisoo et al. (2019).

The PCR and restricted products were visualized on 1% agarose

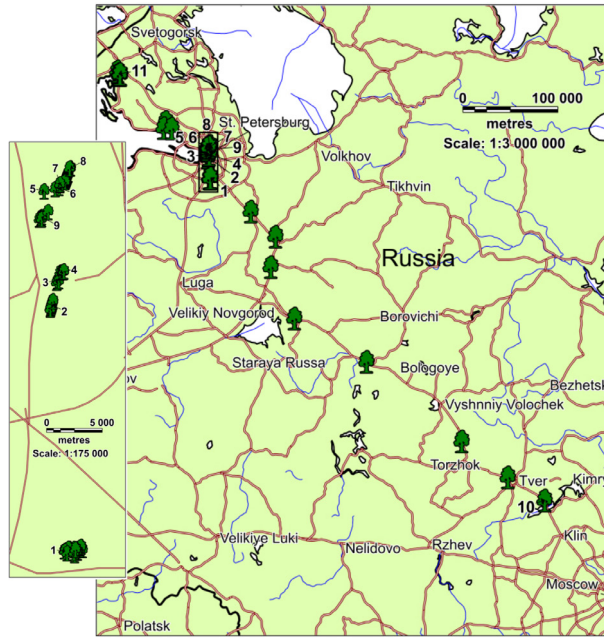

Fig. 1. Sampling plots are numbered and marked by tree shape figures (Pitney Bowes Software, 2015).

Table 1
Number of elm trees analyzed at different sampling sites and types of habitats of north-western Russia.

Habitat	Total number of trees	Surveyed species			
		Ulmus glabra[a]	*U. laevis*	*Ulmus* hybrid	*U. pumila*
Greenspace[b]	102	49	34	19	0
Street	325	100	86	139	1
Nearby highway	234	89	145	0	0
Number of trees	**661**	**237**	**265**	**158**	**1**
Assessed trees (%)	**100**	**36**	**40**	**24**	**0**

[a] Numbers of surveyed trees: *Ulmus glabra* and its cultivar 'Exoniensis' (7 trees).
[b] Green areas, e.g. parks, gardens and squares, courtyards.

(SeaKem® LE Agarose, Lonza) gels under UV light using the Quantum ST4-system (VilberLourmat SAS, Marne-la-allée, France).

ITS-PCR products from isolates from different sites and hosts were sequenced at the Estonian Biocentre in Tartu, using the primer ITS5 (White et al., 1990) and primers F and R for the *col1* gene (Konrad et al., 2002).

The ITS sequences were edited using the BioEdit program, Version 7.2.5 (Hall, 2013) and deposited in Genbank (see Table 3). BLAST searches for the fungal taxa confirmation were performed in the GenBank database (NCBI). All the pure cultures were deposited to the Tartu Fungal Culture Collection (TFC); the data are available in the data management and publishing platform PlutoF (https://plutof.ut.ee/).

3.1. Growth rate of isolates

In the experiment with isolates gathered for the current study, to calculate the growth rate during eleven days for *O. novo-ulmi* both subspecies and their two different hybrids (3 different isolates of each, totally 12 isolates) were used. Each isolate inoculum was placed at the centre of Petri dishes containing 2% Malt Extract Agar (MEA) in three replicates and incubated in darkness at 21°C. Radial growth measurements of fungal colonies (Brasier and Webber, 1987; Tziros et al., 2017) were recorded after four, seven and eleven days from the edge of the initial inoculum in four directions. Measurements were finished on the eleventh day prior to the fungal mycelia reached the Petri dish wall in any dish (Miyashira et al., 2010). The average daily fungal growth rate was calculated for each strain of *O. novo-ulmi* subspecies and their hybrids and expressed as cm per day.

4. Statistical analyses

Statistical analyses and the Mann-Whitney test were carried out to evaluate the impact of different pathogen varieties on the health of elms in different habitats (rural *versus* urban area and street, highway *versus*

Table 2
Minimum, maximum, and mean temperatures and precipitation for the year 2016 and for the periods 1961-1987, 1988-2006 and 2007-2016, including vegetation periods, from three meteorological stations near sampling sites.

Meteorological station	Year and periods	Air temperature (°C) mean				Precipitation sum (mm)	
		annual min.	annual max.	annual	vegetation period[a]	annual	vegetation period[a]
St. Petersburg	2016	−11,2	19,0	6,5	13,1	863,6	531,5
	1961–1987	−17,9	22,2	4,9	12,9	643,2	394,5
	1988–2006	−12,0	21,8	6,0	13,3	643,6	401,0
	2007–2016	−12,1	24,4	6,7	13,6	704,9	423,3
Vyborg	2016	−13,9	18,0	5,4	15,2	730,8	370,9
	1961–1987	−20,0	21,4	3,9	12,9	714,8	412,2
	1988–2006	−14,9	21,1	5,0	13,0	652,2	369,3
	2007–2016	−14,9	23,3	5,6	14,0	756,8	434,6
Staritsa (Tver)	2016	−12,1	18,8	5,3	14,0	773,5	478,4
	1961–1987	−19,6	20,7	3,9	13,1	653,9	413,0
	1988–2006	−11,3	21,2	4,9	12,9	664,5	448,5
	2007–2016	−16,0	23,5	5,5	13,6	697,7	443,9

[a] The part of the year when the daily average temperature exceeds +5°C.

park).

The mean radial growth rate of each isolate was calculated from the three replicate plates.

Dispersion analyses (R Core Team, 2019) was used to evaluate the statistical significance of differences in fungal growth rate among the different strains.

5. Results

5.1. Dutch elm disease agents on hosts and at different habitats

In total, 661 trees (Table 2) were assessed at 61 different sampling sites (11 plots).

Fungal isolations were attempted from 108 symptomatic elm trees (see Materials and Methods) growing on the 11 sampling plots, which resulted in 51 isolates (pure cultures) of *O. novo-ulmi* as identified by ITS region sequencing and species-specific PCR primers. The other fungal species which were also isolated and sequenced from primary pure cultures are shown in Appendix A. *Col1* gene sequencing and restriction with *Bfa* I enzyme provided the first confirmation of the occurrence of both known *O. novo-ulmi* subspecies in Russia (see Table 3). Based on ITS sequencing, none of the isolates were *O. ulmi*. *Cu* and *col1* genes revealed occurrence of hybrids of both *O. novo-ulmi* subspecies. *O. novo-ulmi* subsp. *novo-ulmi* was found in all sampling plots except on the highway from St. Petersburg to Moscow (plot 10; Fig. 1).

Out of a total of 51 isolates, 24 were determined to be *O. novo-ulmi* subsp. *novo-ulmi* and five isolates belonged to subsp. *americana.* All the remaining 22 isolates were identified as hybrids between the subspecies. Seventeen of these strains were hybrids between subsp. *novo-ulmi* and subsp. *americana*. Five isolates of hybrid strains were identified as subsp. *americana* and subsp. *novo-ulmi* (Table 3).

Ophiostoma novo-ulmi subsp. *americana* was found in sampling plots 3, 6, 8 and 9 (Fig. 1). Hybrid *O. novo-ulmi* subsp. *novo-ulmi* x *americana* was found in all sampling plots except plot 6, and the hybrid *O. novo-ulmi* subsp. *americana* x *novo-ulmi* was found in sampling plots 3, 4, 6, 8 and 10.

5.2. Health condition of different elm species in north-western Russia

At all sampling plots, the dieback or death of elm trees was caused by DED. Most of the assessed elm trees belonged to vitality class 2 (Fig. 2).

Among all assessed trees (N = 661) young hybrid elms (<25 years old) showed significantly ($p < 0.001$) higher vitality than older (>40 years old) natural species *U. laevis* and *U. glabra* (see Table 3). Most hybrid elms (91 %) are younger trees and were rated as vitality classes 1 and 2, while 74 % of *U. laevis* and only 56 % of *U. glabra* trees belonged to vitality classes 1 and 2. Twenty percent of *U. glabra*, 5% of *U. laevis* and 4% of hybrid elm trees were found dead (Fig. 2). Mortality of the estimated hybrid elm and *U. laevis* trees was similar, 4–5 %.

The health of *U. glabra* was significantly ($p = 0.03$) better on streets compared to the greenspaces. Overall, the health of *U. glabra* was significantly better ($p < 0.0001$) in urban areas (greenspaces or streets) versus neighboring the highways, and as in the last habitat there were significant groups of dead trees (vitality class 5).

The health status of *U. laevis* was almost the same when comparing them on streets versus greenspaces ($p > 0.05$), and greenspaces versus highways ($p > 0.05$).

The health of hybrid elms was similar ($p > 0.05$) along streets and greenspaces. Generally, the trees were much healthier in greenspaces than along streets or highways; the health condition of hybrid elms on streets was significantly better than *U. laevis* ($p < 0.0001$).

Correlation between symptoms of DED and vitality class of elms showed that from 89 to 100% of trees without DED symptoms were in a higher vitality class (1, 2, or 3), whereas only 46–72 % of DED-symptomatic elm trees were in a higher vitality class.

The meteorological data are not significantly different between the sampling plots 1 and 11.

5.3. Growth rates of DED agents in pure culture

Ophiostoma novo-ulmi subspecies and their hybrids demonstrated significantly ($p < 0.0001$) different growth rate *in vitro* (see Fig. 3). The non-hybridized subspecies *novo-ulmi* and *americana* grew the slowest, 0.46 and 0.47 cm per day, respectively, and not significantly different ($P > 0.05$). Significantly ($P < 0.05$) fastest mycelial growth occurred in *O. novo-ulmi* subsp. *americana* x *novo-ulmi,* with a mean radial growth rate 0.63 cm per day followed by *O. novo-ulmi* subsp. *novo-ulmi* x *americana*, 0.54 cm per day.

5.4. Insects on assessed trees

All the assessed trees (N = 661) were examined for signs of elm bark beetles, e.g., entrance holes, larval galleries etc. Only ten trees of *U. glabra* and 11 of *U. laevis* had attempts or entrance holes of bark beetles and the species were unknown. Twenty-one hybrid elms had entrance holes of *S. pygmaeus*. Larval galleries were not found on most trees, except on dead trees along the highway, where *S. scolytus* and *S. multistriatus* were found on ten trees. Six of those trees were colonized jointly by both indicated beetle species, but for four trees only by *S. multistriatus*. Another 11 dead trees were without signs of bark beetle colonization. Attempted bark beetle attacks were found on two trees in vitality class 4, but the beetle species were not identified since the galleries were on the stems above 2 m.

Table 3
Origin of isolates from symptomatic samples of different habitats and elm species, and molecularly detected DED agents.

Plot	Sampling			Host		Identification No	Tested isolates of SSPP	Molecular identification of *Ophiostoma novo-ulmi* subsp.		Fungal culture collection code[b]	GenBank accession number by ITS
No	date	Site	habitat	species	age, years		*Ophiostoma novo-ulmi*	by *col1*	by *cu+Hph*I		
1	22.07.2016	urban space	street	*U. glabra*	40–80	7037	+	*novo-ulmi*	*novo-ulmi*	TFC101080	MK630338
1	22.07.2016	urban space	street	*U. glabra*	40–80	7038	+	*novo-ulmi*	*novo-ulmi*	TFC101081	
1	22.07.2016	urban space	park	*U. glabra*	40–80	7039	n.d.	*novo-ulmi*	*americana*	TFC101082	
1	22.07.2016	urban space	park	*U. glabra*	40–80	7040	+	*novo-ulmi*	*americana*	TFC101083	
1	22.07.2016	urban space	park	*U. glabra*	40–80	7042	+	*novo-ulmi*	*novo-ulmi*	TFC101085	
1	22.07.2016	urban space	park	*U. glabra*	40–80	7043	+	*novo-ulmi*	*novo-ulmi*	TFC101086	
1	22.07.2016	urban space	park	*U. glabra*	40–80	7044	+	*novo-ulmi*	*novo-ulmi*	TFC101087	MK630340
2	11.06.2016	urban space	street	*U.* hybrid [a]	<25	6771	n.d.	*novo-ulmi*	*novo-ulmi*	TFC101088	
2	11.06.2016	urban space	street	*U.* hybrid [a]	<25	6776	n.d.	*novo-ulmi*	*americana*	TFC101089	
2	11.06.2016	urban space	street	*U.* hybrid [a]	<25	6777	n.d.	*novo-ulmi*	*americana*	TFC101090	
2	11.06.2016	urban space	street	*U.* hybrid [a]	<25	7622	n.d.	*novo-ulmi*	*novo-ulmi*	TFC101091	
2	11.06.2016	urban space	street	*U.* hybrid [a]	<25	7539	n.d.	*novo-ulmi*	*novo-ulmi*	TFC101092	
2	11.06.2016	urban space	street	*U.* hybrid [a]	<25	6796	+	*novo-ulmi*	*novo-ulmi*	TFC101093	MK630331
3	21.07.2016	urban space	street	*U. glabra*	>80	7004	+	*americana*	*americana*	TFC101094	MK630334
3	21.07.2016	urban space	park	*U. laevis*	>80	7005	+	*novo-ulmi*	*americana*	TFC101095	
3	21.07.2016	urban space	park	*U.* hybrid [a]	<25	7007	+	*americana*	*americana*	TFC101096	MK630335
3	21.07.2016	urban space	park	*U.* hybrid [a]	<25	7008	+	*novo-ulmi*	*novo-ulmi*	TFC101097	
4	21.07.2016	urban space	park	*U.* hybrid [a]	<25	7010	+	*novo-ulmi*	*americana*	TFC101098	
4	21.07.2016	urban space	park	*U.* hybrid [a]	<25	7011	+	*americana*	*novo-ulmi*	TFC101099	
4	21.07.2016	urban space	park	*U. laevis*	>80	6868	n.d.	*novo-ulmi*	*novo-ulmi*	TFC101100	
4	21.07.2016	urban space	park	*U.* hybrid [a]	<25	7012	+	*novo-ulmi*	*novo-ulmi*	TFC101101	MK630336
4	21.07.2016	urban space	park	*U.* hybrid [a]	<25	7013	+	*novo-ulmi*	*americana*	TFC101102	
4	21.07.2016	urban space	park	*U.* hybrid [a]	<25	7014	+	*novo-ulmi*	*americana*	TFC101103	
5	21.07.2016	urban space	street	*U. glabra*	40–80	7015	+	*novo-ulmi*	*novo-ulmi*	TFC101104	
5	21.07.2016	urban space	street	*U. glabra*	40–80	7017	+	*novo-ulmi*	*americana*	TFC101105	
5	21.07.2016	urban space	street	*U. glabra*	40–80	7018	+	*novo-ulmi*	*novo-ulmi*	TFC101106	
5	21.07.2016	urban space	street	*U. glabra*	40–80	7019	+	*novo-ulmi*	*novo-ulmi*	TFC101107	
6	21.07.2016	urban space	street	*U. glabra*	>80	7020	+	*novo-ulmi*	*novo-ulmi*	TFC101108	
6	11.06.2016	urban space	street	*U. glabra*	>80	6858	+	*americana*	*novo-ulmi*	TFC101109	MK630330
6	21.07.2016	urban space	street	*U. glabra*	>80	7034	+	*americana*	*americana*	TFC101110	MK630337
6	21.07.2016	urban space	street	*U. glabra*	>80	7036	+	*novo-ulmi*	*novo-ulmi*	TFC101111	
7	21.07.2016	urban space	street	*U. glabra*	>80	7021	+	*novo-ulmi*	*americana*	TFC101112	
7	21.07.2016	urban space	street	*U. glabra*	>80	6965	+	*novo-ulmi*	*novo-ulmi*	TFC101113	
7	21.07.2016	urban space	street	*U. glabra*	>80	7028	+	*novo-ulmi*	*novo-ulmi*	TFC101114	MK630339
7	21.07.2016	urban space	street	*U. glabra*	>80	7030	n.d.	*novo-ulmi*	*americana*	TFC101115	
7	21.07.2016	urban space	street	*U. glabra*	>80	7031	+	*novo-ulmi*	*americana*	TFC101116	
8	10.06.2016	urban space	street	*U. laevis*	40–80	6857	+	*novo-ulmi*	*americana*	TFC101117	MK561564
8	10.06.2016	urban space	street	*U. laevis*	40–80	6787	+	*novo-ulmi*	*novo-ulmi*	TFC101118	
8	10.06.2016	urban space	street	*U. laevis*	40–80	6859	+	*americana*	*americana*	TFC101119	MK630328
8	10.06.2016	urban space	street	*U. laevis*	40–80	6789	+	*novo-ulmi*	*novo-ulmi*	TFC101120	
8	10.06.2016	urban space	street	*U. laevis*	40–80	6792	n.d.	*americana*	*novo-ulmi*	TFC101122	MK630329
9	10.06.2016	urban space	park	*U. laevis*	>80	6779	+	*novo-ulmi*	*americana*	TFC101123	
9	10.06.2016	urban space	park	*U. laevis*	>80	6780	+	*novo-ulmi*	*novo-ulmi*	TFC101124	MK561563
9	10.06.2016	urban space	park	*U. glabra*	>80	6862	+	*novo-ulmi*	*americana*	TFC101125	MK630327
9	10.06.2016	urban space	park	*U. laevis*	>80	6783	+	*americana*	*americana*	TFC101126	
9	14.06.2016	urban space	park	*U. glabra*	>80	7618	+	*novo-ulmi*	*novo-ulmi*	TFC101127	
10	02.07.2016	rural	highway	*U. glabra*	40–80	6828	+	*americana*	*novo-ulmi*	TFC101128	MK630332
10	02.07.2016	rural	highway	*U. glabra*	40–80	6829	+	*americana*	*novo-ulmi*	TFC101129	MK630333
10	02.07.2016	rural	highway	*U. glabra*	40–80	6824	+	*novo-ulmi*	*americana*	TFC101130	
11	23.07.2016	urban space	street	*U. glabra*	>80	7045	n.d.	*novo-ulmi*	*americana*	TFC101131	
11	23.07.2016	urban space	street	*U. glabra*	>80	7046	+	*novo-ulmi*	*novo-ulmi*	TFC101132	MK630341

Identification No. – in the collection of the Laboratory of Forest Pathology and Genetics of the Estonian University of Life Sciences.
SSPP – species-specific PCR primers (Gibb and Hausner, 2005) were used to detect *Ophiostoma novo-ulmi* or *O. ulmi* for all the isolates.
col1 – the colony type gene (Konrad et al., 2002); the sequence similarity threshold ≥ 99 % for *Ophiostoma novo-ulmi* subspecies detection.
cu+Hph I – ceratoulmin gene; RFLP banding pattern of *cu* from *O. novo-ulmi* digested with enzyme *Hph* I (Konrad et al., 2002).
ITS – internal transcribed spacer; the sequence similarity threshold ≥ 99 % for *Ophiostoma* spp. detection.
GenBank (NCBI- https://www.ncbi.nlm.nih.gov/).
n.d. - not detected.
[a] Precise elm cultivar is unknown.
[b] Tartu Fungal Collection in Estonian University of Life Sciences, Estonia (TFC).

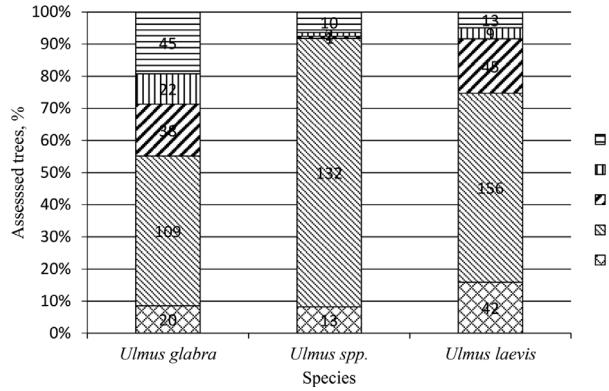

Fig. 2. The percentage of assessed elm species in different vitality classes (ranging from 1=healthy to 5=dead). *Ulmus* spp. means all hybrid elms together.

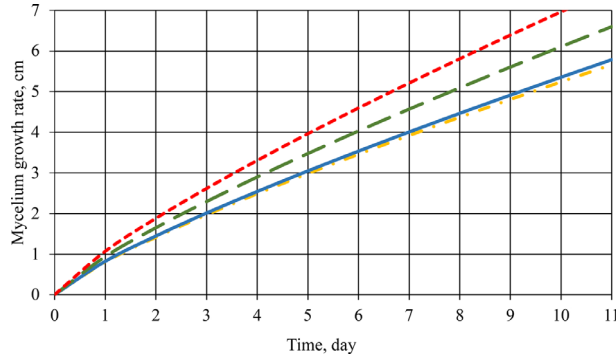

Fig. 3. The radial growth rate of isolates of different agents of DED. Lines: continuous (blue) – *O. novo-ulmi* subsp. *novo-ulmi,* dash-dotted (yellow) – *O. novo-ulmi* subsp. *americana,* dashed (green) – hybrid *O. novo-ulmi* subsp. *novo-ulmi* x *americana* and dotted (red) – hybrid *O. novo-ulmi* subsp. *americana* x *novo-ulmi.* (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

6. Discussion

6.1. DED in north-western part of Russia

This study represents the first records of both subspecies of the invasive pathogen *Ophiostoma novo-ulmi*: *O. novo-ulmi* subsp. *novo-ulmi* and *O. novo-ulmi* subsp. *americana,* as well as hybrids of the two subspecies, in north-western Russia identified by using molecular methods.

According to the global database published by the European and Mediterranean Plant Protection Organization (EPPO Global Database, 2019) *Ophiostoma ulmi* has been reported since 1979 in the European part of Russia. Actually, in the second half of the last century, *O. novo-ulmi* was considered to be widespread in southern Russia (Gibbs, 1978) and *O. novo-ulmi* subsp. *novo-ulmi* was detected there later (Brasier and Kirk, 2001a, 2001b). Phytopathological studies of DED showed that the causative agents in St. Petersburg were *O. ulmi* and *O. novo-ulmi* (Kalko, 2008), but in the current work we did not find any isolates attributable to the species *O. ulmi.*

Ophiostoma novo-ulmi arrived later, but the subspecies *americana* and *novo-ulmi* may have been distributed differently and separately (Brasier and Kirk, 2001a, 2001b). In the current work, we recorded the presence of both subspecies as well as the hybrids among them in north-western Russia. Based on growth rates *in vitro*, these hybrids may be more virulent (Brasier and Afsharpour, 1979; Brasier and Webber, 1987; Gibbs and Brasier, 1973; Gibbs et al., 1975). However, the higher pathogenicity of the pathogen hybrids can be proved by the inoculation tests (Brasier and Afsharpour, 1979; Gibbs et al., 1975; Santini et al., 2005a), but this was not the aim of this particular work.

Reports of hybrid fungi were quite rare until the 1990s (Brasier, 1995; Brasier and Buck, 2001). Indirectly, hybridization is promoted by

international movement of plants and through plant nurseries (Brasier, 2012, 2008; Wingfield et al., 2015), by weakened hosts (Brasier, 1995), by environmental disturbance incl. breeding and by evolutionary opportunities (Brasier et al., 2004). Hybridization is directly supported by immigration of fungal pathogens into new areas, and weak genetic barriers between invasive and resident pathogens (Brasier, 2012). Because subspecies of *O. novo-ulmi* overlap in Europe (Martín et al., 2010) and gene flow between them lacks strong barriers (Brasier and Buck, 2001), they are hybridizing freely (Santini et al., 2005b; Tziros et al., 2017), as occurred in a horticultural nursery in Europe (Brasier et al., 2004). It could also be possible that all potential pathogen hybrids could not be detected using only *cu* and *col1* genes (Tziros et al., 2017). Thus, new and reliable studies are needed to analyse the population structure of DED agents in north-eastern Europe, including potential hybrids.

6.2. Elm bark beetles in St. Petersburg

In the area of St. Petersburg and in the southern Leningrad Region several species of elm bark beetles were reported – *Scolytus scolytus* (Obert, 1876), and also *Scolytus multistriatus* and *Scolytus laevis* Chapuis (Mandelshtam and Khairetdinov, 2017; Shcherbakova and Mandelshtam, 2014). During the first half of the last century no species of elm bark beetles have been mentioned in the special review of important pests of trees in St. Petersburg (Venkova and Zanadvorova, 1939). Lack of DED insect vectors could be a reason why elms remained unaffected (Martín et al., 2019; Santini and Faccoli, 2013) by the disease before the last decade of the previous century. The outbreak of elm bark beetles started from the southern suburb of St. Petersburg (Pushkin) in 1995 (Selikhovkin et al., 2010; Shcherbakova, 2008). *Scolytus multistratus* had been observed there since 1998 and *S. scolytus* since 2001 (Dorofeeva, 2008), but *S. pygmaeus* has been common since 2012 (Selikhovkin et al., 2014; Shcherbakova and Mandelshtam, 2014). Most likely, *S. pygmaeus*, a known vector of DED in Europe (Basset et al., 1992), had arrived with planting material because it was first found in several newly-planted green areas (Shcherbakova and Mandelshtam, 2014). *Scolytus scolytus* and *S. multistriatus* migrated northward from areas of natural distribution (Mandelshtam and Khairetdinov, 2017; Shcherbakova and Mandelshtam, 2014). It is quite probable that climate change is increasing the occurrence of warming, creating more drought or wet periods (Hanso and Drenkhan, 2013), which support the spread of pathogens and also their insect vectors to northwards (see Caulton et al., 1998; Drenkhan et al., 2020; Hanso and Drenkhan, 2012; Selikhovkin et al., 2020). Currently elm bark beetles are widespread in greenspaces in the cities of Russia, including St. Petersburg (Mandelshtam and Popovichev, 2000; Shcherbakova, 2008). The DED epidemic in St. Petersburg may be due to a high number of bark beetles and the result is high disease pressure on elms. The results show that all these bark beetles are present in the sampled elm stands. Bark beetles were not found in all samples, but it should be noted that we were unable to survey trees at a height of more than two meters. It was very likely that some of them could feed on twigs and shoots in the upper part of the crowns as elm bark beetles often do maturation feeding in twig crotches of healthy elms (Menkis et al., 2016). Native *U. laevis* is significantly healthier than *U. glabra*, because *U. laevis* is less attractive to bark beetles (Mittempergher et al., 1993; Sacchetti et al., 1990). *Ulmus glabra* and *U. minor* are similarly attractive to bark beetles and both are strongly affected by DED (Solla et al., 2005b).

Mass trapping of vector beetles is also one possibility to slow down the spread of DED, but that is effective only if there are isolated bark beetle populations (El-Sayed et al., 2006). Sanitation activity to destroy DED diseased and bark beetle infested elms from green areas would be more effective control measure in the case of St. Petersburg and southern Leningrad Region.

6.3. Health condition of elms

This study indicates that the main reason for the massive death of elms there was likely the spread of different agents of DED, incl. hybrids. The health of *U. glabra* was worse compared to the other elm species at all habitats and sampling sites in north-west Russia (Figs. 1 and 2). In plot 1 all *U. glabra* trees were affected by DED, suggesting that the disease may have started from that site (Selikhovkin et al., 2010). In this sampling plot (Pushkin), infected elm trees (N = 28) were found everywhere, but only a single, old *U. laevis* was found to be asymptomatic there. In the park of the State Forest Technical University in St. Petersburg (plot 9) about 70 % of elms died during 1995–2014 (A. Selikhovkin, pers. comm.). In this assessment only 16 *U. laevis* and 12 *U. glabra* trees were found in the park of the State Forest Technical University in 2016. For example, in plot 11 (Primorskaya and Zelenogorsk) were free of DED, which is 70 km from plot 9. However, about 100 km from plot 9 two symptomatic trees were found close to the Finnish border in Vyborg (plot 11). DED has not yet been discovered in Finland (Hannunen and Marinova-Todorova, 2016; Hantula, 2021).

Close to the highway from St. Petersburg to Moscow, *U. glabra* suffered more than *U. laevis*. The same situation was traceable with *U. glabra* versus *U. laevis* in the arboretum of the botanical garden next to plot 9 (Firsov and Bulgakov, 2017) as in other areas in Europe (Napierała-Filipiak et al., 2016). Elms are tolerant species and can survive in nearly all environments (Mackenthun, 2007), and also tolerate contact with salt (Šerá, 2017), but it seems that polluted conditions next to highways are not suitable for representatives of *Ulmus*. We also collected several samples from the elm cultivars, which could be two hybrids: *Ulmus davidiana* var. *japonica* × *U. pumila* 'New Horizon' or *Ulmus glabra* `Exoniensis' x *U. wallichiana* 'Dodoens'. There are many kilometres of allées of native as well as hybrid elms in the St. Petersburg area. One long allée contained almost 150 specimens of an elm cultivar; several of those trees had been cut off and some of the remaining trees had symptoms of DED. All of the elm cultivars planted during this period were not resistant to DED. Latter selections of elms are mostly bred to be resistant to DED (Pecori et al., 2017). Thus, comparisons of different elm cultivars show a large variety of DED-resistance whether the parents are native or Asian species (Buiteveld et al., 2015; Santini et al., 2005a; Solla et al., 2005a). Environmental conditions including climate change events weaken the hosts, and consequently the elm cultivars may increase susceptibility to DED under high disease pressure (Buiteveld et al., 2015). In the current work, some analysed elm cultivars that had typical DED symptoms and pathogens were isolated and molecularly confirmed from these hosts (see Table 3).

We have to consider that in north-westernmost Russia even *Ulmus glabra* and *U. laevis* grow close to the northern limit of their natural ranges (Ignatieva et al., 2011). Thus, elms of foreign provenance may be sensitive to local climatic conditions (Bowring et al., 2009), as also seen in Estonia, in the northern Baltic (see Jürisoo et al., 2019). The climates on different continents are never similar even if the latitude is the same (König, 2005), and thus geographical provenance trials are recommended for sensitive introduced plants.

Before cities expand their plant assortments with new varieties of hybrid elms they need to consider if one of the hybrid elm parents could be *U. pumila*, a species adapted to a continental climate which may suffer from late frosts in spring (Firsov and Fadeeva, 2014). In this study it is difficult to recommend suitable hybrid elms for north-western Russia, because the precise elm cultivars and origins are unknown.

The situation of elms in the Leningrad Region is extraordinarily worrisome and there has to be a plan for the future. The spread of DED can be slowed with the help of a sanitation program involving removal of affected trees (Solheim et al., 2011). To protect disease free areas transport of elm wood from DED-affected areas should be prohibited (Solheim et al., 2011). The introduced planting material should be controlled and certified (Martín et al., 2019). It is recommended to test native (e.g. susceptible *U. glabra*) as well as new elm cultivars under

local conditions before using them in urban green areas, e.g. in north-eastern Europe (Buiteveld et al., 2015). Breeding programs tests for resistant elms in different countries are usually based on local conditions (Santini et al., 2010), or local origin planting material such as *Ulmus laevis* could be used as an alternative as it is less attractive to the vectors than other European elms (Sacchetti et al., 1990).

7. Conclusions

The massive, intensive arrival and reproduction of destructive agents for trees in northern Europe is related to climate change as well as the intensification of international trade of plant materials. There have been over 5000 deaths of elms throughout greenspaces in the north-western Russia. This study represents the first records of Dutch elm disease (DED) agents confirmed using molecular methods, two subspecies, as being the invasive pathogens – *O. novo-ulmi* subsp. *novo-ulmi* and *O. novo-ulmi* subsp. *americana* and hybrids of those pathogens. The presence of different DED agents and their hybrids as well as insect vectors may be one cause of the massive death of elms in north-western Russia.

Some recommendations from the current work are listed below:

- Disease monitoring can be part of an effective sanitation program.
- For assessing the composition and abundance of DED vectors, pheromone traps could be used, and possibly for mass trapping.
- It is recommended to use either local native *U. laevis* planting material or suitable resistant elm cultivars more than *U. glabra* in different habitats.
- If there is an interest to use elms of new origin, then is recommended to test these in geographical provenance trials before planting them in north-eastern Europe.
- Different, identified DED agent strains for native and hybrid elms using comparative inoculation tests should be conducted to select resistant elm species or origins.
- Introduced planting material should be certified and controlled to avoid spreading non-native pathogens.

Author statement

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Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. List of other isolated and determined fungi from different elm trees' shoots

No	Sampling				Host species	Molecular identification
	plot	date	location	habitat		
1	9	10.06.2016	urban	park	*Ulmus laevis*	*Phomopsis* sp.
2	9	10.06.2016	urban	park	*U. glabra*	*Dothiora maculans*
3	2	11.06.2016	urban	street	*Ulmus* hybrid [1]	*Diaporthe* sp.
4	2	11.06.2016	urban	street	*Ulmus* hybrid [1]	*Phoma* sp.
5	2	11.06.2016	urban	street	*Ulmus* hybrid [1]	*Aureobasidium pullulans*
6	2	11.06.2016	urban	street	*Ulmus* hybrid [1]	*Phoma* sp.
7	2	11.06.2016	urban	street	*Ulmus* hybrid [1]	*Aureobasidium pullulans*
8	2	11.06.2016	urban	street	*Ulmus* hybrid [1]	*Aureobasidium pullulans*
9	2	11.06.2016	urban	street	*Ulmus* hybrid [1]	*Aureobasidium pullulans*
10	2	11.06.2016	urban	street	*Ulmus* hybrid [1]	*Aureobasidium pullulans*
11	10	02.07.2016	rural	highway	*U. glabra*	*Phaebotryon sp.*
12	10	02.07.2016	rural	highway	*U. glabra*	*Aureobasidium pullulans*
13	11	23.07.2016	urban	street	*U. glabra*	*Phomopsis* sp.

[1]Precise elm cultivars are unknown.

References

Adamson, K., Drenkhan, R., Hanso, M., 2015a. Invasive brown spot needle blight caused by *Lecanosticta acicola* in Estonia. Scand. J. For. Res. 30, 587–593. https://doi.org/10.1080/02827581.2015.1041550.

Adamson, K., Klavina, D., Drenkhan, R., Gaitnieks, T., Hanso, M., 2015b. *Diplodia sapinea* is colonizing the native Scots pine (*Pinus sylvestris*) in the northern Baltics. Eur. J. Plant Pathol. 143, 343–350. https://doi.org/10.1007/s10658-015-0686-8.

Adamson, K., Laas, M., Drenkhan, R., Hanso, M., 2018a. Quarantine pathogen *Lecanosticta acicola*, observed at its jump from an exotic host to the native Scots pine in Estonia. Balt. For. 24, 36–41.

Adamson, K., Mullett, M.S., Solheim, H., Barnes, I., Müller, M.M., Hantula, J., Vuorinen, M., Kačergius, A., Markovskaja, S., Musolin, D.L., Davydenko, K., Keča, N., Ligi, K., Priedite, R.D., Millberg, H., Drenkhan, R., 2018b. Looking for relationships between the populations of *Dothistroma septosporum* in northern Europe and Asia. Fungal Genet. Biol. 110, 15–25. https://doi.org/10.1016/J.FGB.2017.12.001.

Agan, A., Drenkhan, R., Adamson, K., Tedersoo, L., Solheim, H., Børja, I., Matsiakh, I., Timmermann, V., Nagy, N.E., Hietala, A.M., 2020. The relationship between fungal diversity and invasibility of a foliar niche—the case of ash dieback. J. Fungi 6, 1–27. https://doi.org/10.3390/jof6030150.

Basset, Y., Favaro, A., Springate, N.D., Battisti, A., 1992. Observations on the relative effectiveness of *Scolytus multistriatus* (Marsham) and *Scolytus pygmaeus* (Fabricius) (Coleoptera : Scolytidae) as vectors of the Dutch elm disease [In German]. Mitteilungen der Schweizerischen Entomol. Gesellschaft 65, 61–67.

Bowring, J., Egoz, S., Ignatieva, M., 2009. 'As good as the West': two paradoxes of globalisation and landscape architecture in St. Petersburg. J. Jpn. Landsc. Archit. Soc. 4, 6–15. https://doi.org/10.1080/18626033.2009.9723409.

Brasier, C.M., 1995. Episodic selection as a force in fungal microevolution, with special reference to clonal speciation and hybrid introgression. Can. J. Bot. 73, 1213–1221. https://doi.org/10.1139/b95-381.
Brasier, C.M., 2008. The biosecurity threat to the UK and global environment from international trade in plants. Plant Pathol. 57, 792–808. https://doi.org/10.1111/j.1365-3059.2008.01886.x.
Brasier, C.M., 2012. Rapid evolution of introduced tree pathogens via episodic selection and horizontal Gene transfer. In: Sniezko, R.A., Yanchuk, A.D., Kliejunas, J.T., Palmieri, K.M., Alexander, J.M., Frankel, S.J. (Eds.), Proceedings of the 4th International Workshop on Genetics of Host-Parasite Interactions in Forestry. Pacific Sothwest Research Station, Forest Service, U.S. Department of Agriculture., Albany, CA, pp. 133–142.
Brasier, C.M., Afsharpour, F., 1979. The aggressive and non-aggressive strains of *Ceratocystis ulmi* in Iran. Eur. J. Forest Pathol. 9, 113–122. https://doi.org/10.1111/j.1439-0329.1979.tb00668.x.
Brasier, C.M., Buck, K.W., 2001. Rapid evolutionary changes in a globally invading fungal pathogen (Dutch Elm Disease). Biol. Invasions 3, 223–233. https://doi.org/10.1023/A:1015248819864.
Brasier, C.M., Kirk, S.A., 2001a. Designation of the EAN and NAN races of *Ophiostoma novo-ulmi* as subspecies. Mycol. Res. 105, 547–554. https://doi.org/10.1017/S0953756201004087.
Brasier, C.M., Kirk, S.A., 2001b. Designation of the EAN and NAN races of *Ophiostoma novo-ulmi* as subspecies. Mycol. Res. 105, 547–554. https://doi.org/10.1017/S0953756201004087.
Brasier, C.M., Kirk, S.A., 2010. Rapid emergence of hybrids between the two subspecies of *Ophiostoma novo-ulmi* with a high level of pathogenic fitness. Plant Pathol. 59, 186–199. https://doi.org/10.1111/j.1365-3059.2009.02157.x.
Brasier, C.M., Webber, J.F., 1987. Positive correlations between in vitro growth rate and pathogenesis in *Ophiostoma ulmi*. Plant Pathol. 36, 462–466. https://doi.org/10.1111/j.1365-3059.1987.tb02263.x.
Brasier, C.M., Buck, K., Paoletti, M., Crawford, L.J., Kirk, S., 2004. Molecular analysis of evolutionary changes in populations of *Ophiostma novo-ulmi*. Investig. Agrar. Sist. Y Recur. For. 13, 93–103. https://doi.org/10.5424/817.
Buiteveld, J., Van Der Werf, B., Hiemstra, J.A., 2015. Comparison of commercial elm cultivars and promising unreleased Dutch clones for resistance to *Ophiostoma novo-ulmi*. iForest - Biogeosciences For. 8, 158–164. https://doi.org/10.3832/ifor1209-008.
Bulygina, O.N., Razuvayev, V.N., Aleksandrova, T.M., Razuvayev, V.N., Aleksandrova, T. M., 2019. 2019. Description of the data set of the atmospheric temperature and rainfall at the meteorological stations of Russia and the former USSR (Opisaniye massiva dannykh sutotsnoy temperatury vozdukha i kolitshestva osadkov na meteorologicheskikh stantsiyakh Rossii [WWW Document]. Fed. Serv. Hydrometeorol. Environ. Monit. URL http://meteo.ru/data/162-temperature-precipitation.
Caulton, E., Aitken, W., Rashid, N., 1998. Aerobiological aspects of elm (*Ulmus* spp) in South-East Scotland in relation to elm decline from Dutch elm disease (1976–1996). Aerobiologia (Bologna) 14, 147–153. https://doi.org/10.1007/BF02694199.
Cleary, M., Laas, M., Oskay, F., Drenkhan, R., 2019. First report of *Lecanosticta acicola* on non-native *Pinus mugo* in southern Sweden. For. Pathol. 49 https://doi.org/10.1111/efp.12507.
Dickie, I.A., Bufford, J.L., Cobb, R.C., Desprez-Loustau, M.-L., Grelet, G., Hulme, P.E., Klironomos, J., Makiola, A., Nuñez, M.A., Pringle, A., Thrall, P.H., Tourtellot, S.G., Waller, L., Williams, N.M., 2017. The emerging science of linked plant-fungal invasions. New Phytol. 215, 1314–1332. https://doi.org/10.1111/nph.14657.
Dorofeeva, T.B., 2008. Dutch elm disease epiphytoty in St. Petersburg (Epifitotija ofiostomoza v Sankt-Peterburge) [in Russian]. Zashita i karantin rasteniy (Protection Quar. plants) 3, 59.
Dorofeeva, T.B., Tyupina, G.N., 2002. Dutch elm disease of the elm trees in St. Petersburg and measures to combat it (Grafioz ilmovyh v Sankt-Peterburge I mery borby s nim) [in Russian]. Ecology of a Large City. Prima-M, Moscow, pp. 57–61.
Drenkhan, R., Sander, H., Hanso, M., 2014. Introduction of Mandshurian ash (*Fraxinus mandshurica*Rupr.) to Estonia: is it related to the current epidemic on European ash (*F. excelsior* L.)? Eur. J. For. Res. 133, 769–781. https://doi.org/10.1007/s10342-014-0811-9.
Drenkhan, R., Adamson, K., Hanso, M., 2015. Fraxinus sogdiana, a central asian ash species, is susceptible to Hymenoscyphus fraxineus. Plant Prot. Sci. 51, 150–152. https://doi.org/10.17221/89/2014-PPS.
Drenkhan, R., Riit, T., Adamson, K., Hanso, M., 2016. The earliest samples of *Hymenoscyphus albidus* vs. *H. fraxineus* in Estonian mycological herbaria. Mycol. Prog. 15, 835–844. https://doi.org/10.1007/s11557-016-1209-5.
Drenkhan, R., Ganley, B., Martín-García, J., Vahalík, P., Adamson, K., Adamcíková, K., Ahumada, R., Blank, L., Bragança, H., Capretti, P., Cleary, M., Cornejo, C., Davydenko, K., Diez, J.J., Lehtijärvi, H.T.D., Dvořák, M., Enderle, R., Fourie, G., Georgieva, M., Ghelardini, L., Hantula, J., Ioos, R., Iturritxa, E., Kanetis, L., Karpun, N.N., Koltay, A., Landeras, E., Markovskaja, S., Mesanza, N., Milenkovic, I., Musolin, D.L., Nikolaou, K., Nowakowska, J.A., Ogris, N., Oskay, F., Oszako, T., Papazova-Anakieva, I., Paraschiv, M., Pasquali, M., Pecori, F., Rafoss, T., Raitelaityte, K., Raposo, R., Robin, C., Rodas, C.A., Santini, A., Sanz-Ros, A.V., Selikhovkin, A.V., Solla, A., Soukainen, M., Soulioti, N., Steenkamp, E.T., Tsopelas, P., Vemic, A., Vettraino, A.M., Wingfield, M.J., Woodward, S., Zamora-Ballesteros, C., Mullett, M.S., 2020. Global geographic distribution and host range of *Fusarium circinatum*, the causal agent of pine pitch canker. Forests. https://doi.org/10.3390/F11070724.
Drobyshev, I.V., 2001. Effect of natural disturbances on the abundance of Norway spruce (*Picea abies* (L.) Karst.) regeneration in nemoral forests of the southern boreal zone. For. Ecol. Manage. 140, 151–161. https://doi.org/10.1016/S0378-1127(00)00324-8.
Dvořák, M., Tomšovský, M., Jankovský, L., Novotný, D., 2007. Contribution to identify the causal agents of Dutch elm disease in the Czech Republic. Plant Prot. Sci. 43, 142–145.
El-Sayed, A.M., Suckling, D.M., Wearing, C.H., Byers, J.A., 2006. Potential of mass trapping for long-term pest management and eradication of invasive species. J. Econ. Entomol. 99, 1550–1564. https://doi.org/10.1093/jee/99.5.1550.
EPPO Global Database [WWW Document], 2019. EPPO. URL https://gd.eppo.int/ (Accessed 7 June 2019).
Fedorova, N.B., 2009. Dutch elm disease of elm plantations on Vasilievsky Island (St. Petersburg) (Gollandskaya bolezn vjazovyh na Vasiljevskom ostrove (Sankt-Peterburg)) [in Russian]. Lesn. Vestnik. (The For. Herald) 5, 161–163.
Firsov, G., Bulgakov, T., 2017. The modern state of elms (*Ulmus* L., Ulmaceae) in arboretum of Peter the Great Botanic Garden under conditions of epiphytoty of the Dutch elm disease. Hortus Bot. 12, 278–312. https://doi.org/10.15393/j4.art.2017.3962.
Firsov, G.A., Fadeeva, I.V., 2014. The Influence of Bioclimatic Cyclicity on Arboreal Plants in Saint Petersburg. (Vliyaniye bioklimaticheskoy tsiklichnosti na drevesnyye rasteniya v Sankt-Peterburge) [In Russian, Abstract in English], 2. Vestn. Volgogr. gossudarstvennogo Univ. Ser. 11, Estestv. Nauk., pp. 18–26
Gardes, M., Bruns, T.D., 1993. ITS primers with enhanced specificity for basidiomycetes - application to the identification of mycorrhizae and rusts. Mol. Ecol. 2, 113–118. https://doi.org/10.1111/j.1365-294X.1993.tb00005.x.
Gibb, E.A., Hausner, G., 2005. Optional mitochondrial introns and evidence for a homing-endonuclease gene in the mtDNA rnl gene in *Ophiostoma ulmi* s. lat. Mycol. Res. 109, 1112–1126. https://doi.org/10.1017/S095375620500376X.
Gibbs, J.N., 1978. Intercontinental epidemiology of Dutch Elm Disease. Annu. Rev. Phytopathol. 16, 287–307. https://doi.org/10.1146/annurev.py.16.090178.001443.
Gibbs, J., Brasier, C.M., 1973. Correlation between cultural characters and pathogenicity in *Ceratocystis ulmi* from Britain, Europe and America. Nature 241, 381–383. https://doi.org/10.1038/241381a0.
Gibbs, J.N., Brasier, C.M., McNabb, H.S., Heybroek, H.M., 1975. Further studies on pathogenicity in *Ceratocystis ulmi*. Eur. J. Forest Pathol. 5, 161–174. https://doi.org/10.1111/j.1439-0329.1975.tb00461.x.
Golovach, A.G., 1980. Derev'ya, kustarniki i liany Botanicheskogo sada Botanicheskogo Instituta, Akademii Nauk SSSR (Trees, schrubs, and lianas of the Botanical Garden, Botanical Intitute, Academy of Sciences of Soviet Union) [In Russian]. Nauka, Leningrad, p. 21.
Hall, T.A., 2013. Biological Sequence Alignment Editor and Analysis Program for Windows 95/98/NT/2000/XP/7 [WWW Document]. BioEdit. URL. http://www.mbio.ncsu.edu/bioedit/bioedit.html.
Hannunen, S., Marinova-Todorova, M., 2016. Pest Risk Assessment for Dutch Elm Disease. Helsinki.
Hanso, M., Drenkhan, R., 2009. *Diplodia pinea* is a new pathogen on Austrian pine (*Pinus nigra*) in Estonia. Plant Pathol. https://doi.org/10.1111/j.1365-3059.2009.02082.x.
Hanso, M., Drenkhan, R., 2012. Lophodermium needle cast, insect defoliation and growth responses of young Scots pines in Estonia. For. Pathol. 42, 124–135. https://doi.org/10.1111/j.1439-0329.2011.00728.x.
Hanso, M., Drenkhan, R., 2013. Simple visualization of climate change for improving the public perception in forest pathology. For. Stud. 58, 37–45. https://doi.org/10.2478/fsmu-2013-0004.
Hantula, J., 2021. The story of Dutch elm disease (Hollaninjalavataudi tarina) [In Finnish]. Sorbifolia 52 (1), 31–35.
Hemery, G.E., Clark, J.R., Aldinger, E., Claessens, H., Malvolti, M.E., O'Connor, E., Raftoyannis, Y., Savill, P.S., Brus, R., 2010. Growing scattered broadleaved tree species in Europe in a changing climate: a review of risks and opportunities. Forestry 1, 65–81. https://doi.org/10.1093/forestry/cpp034.
Hillier Nurseries, 1991. The Hillier Manual of Trees & Shrubs, 6th ed. David & Charles, Winchester.
Hintikka, V., 1974. *Ceratocystis ulmi* in Finland. Karstenia 14, 32. https://doi.org/10.29203/ka.1974.87.
Hulme, P.E., 2009. Trade, transport and trouble: managing invasive species pathways in an era of globalization. J. Appl. Ecol. https://doi.org/10.1111/j.1365-2664.2008.01600.x.
Ignatieva, M., 2005. Case study: heritage landscapes in St. Petersburg, Russia: past and present. In: Abbott, M. (Ed.), Looking Forward to Heritage Landscapes: Proceedings of NZ Institute of Landscape Architects. New Zealand Institute of Landscape Achitects, pp. 337–347.
Ignatieva, M., Konechnaya, G., 2004. Floristic Investigations of Historical Parks in Floristic Investigations of Historical Parks in St. Petersburg, Russia.
Ignatieva, M., Konechnaya, G., Stewart, G., 2011. St. Petersburg. Plants and Habitats of European Cities. Springer, New York, New York, NY, pp. 407–452. https://doi.org/10.1007/978-0-387-89684-7_12.
Jürisoo, L., Adamson, K., Padari, A., Drenkhan, R., 2019. Health of elms and Dutch elm disease in Estonia. Eur. J. Plant Pathol. 154, 823–841. https://doi.org/10.1007/s10658-019-01707-0.
Jürisoo, Liina, Süda, Ilmar, Agan, Ahto, Drenkhan, Rein, 2021. Vectors of Dutch Elm Disease in Northern Europe. Insects 12 (5), 393. https://doi.org/10.3390/insects12050393.
Kalko, G.V., 2008. Dutch Elm Disease in St. Petersburg [In Russian] Gollandskaya bolezn vjazov v Sankt Peterburge. Summary in English. Mycol. Phytopathol. 42, 564–571.
König, A.O., 2005. Provenance research: evaluating the spatial pattern of genetic variation. In: Geburek, T., Turok, J. (Eds.), Conservation and Management of Forest Genetic Resources in Europe. Arbora Publishers, Zvolen, pp. 275–333.

Konrad, H., Kirisits, T., Riegler, M., Halmschlager, E., Stauffer, C., 2002. Genetic evidence for natural hybridization between the Dutch elm disease pathogens *Ophiostoma novo-ulmi* ssp. *novo-ulmi* and *O. novo-ulmi* ssp. *americana*. Plant Pathol. 51, 78–84.

Laas, M., Adamson, K., Drenkhan, R., 2019. A look into the genetic diversity of *Lecanosticta acicola* in northern Europe. Fungal Biol. 123, 773–782. https://doi.org/10.1016/j.funbio.2019.06.012.

Lutter, R., Drenkhan, R., Tullus, A., Jürimaa, K., Tullus, T., Tullus, H., 2019. First record of *Entoleuca mammata* in hybrid aspen plantations in hemiboreal Estonia and stand–environmental factors affecting its prevalence. Eur. J. For. Res. 138, 263–274. https://doi.org/10.1007/s10342-019-01165-7.

Mackenthun, G.L., 2007. The elms of Co Cork - a survey of species, varieties and forms. Irish For. 64, 44–60.

Mandelshtam, M.Y., Khairetdinov, R.R., 2017. Additions to the check list of bark beetles (Coleoptera, Curculionidae: Scolytinae) from Leningrad Province, Russia. Entomol. Rev. 97, 893–899. https://doi.org/10.1134/S0013873817070053.

Mandelshtam, M.Y., Popovichev, B.G., 2000. Annotated list of bark beetle species (Coleoptera, Scoytidae) of the Leningrad Region. Entomol. Rev. 80, 887–903.

Markova, T., 2000. Why do elms and oaks die? (Pochemu gibnut vyazy i duby?) [in Russian]. Tsarskoselskaya Newsp. 103 (9128).

Martín, J.A., Fuentes-Utrilla, P., Gil, L., Witzell, J., 2010. Ecological factors in Dutch elm disease complex in Europe - a review. Ecol. Bull. 53, 209–224.

Martín, J.A., Sobrino-Plata, J., Rodríguez-Calcerrada, J., Collada, C., Gil, L., 2019. Breeding and scientific advances in the fight against Dutch elm disease: will they allow the use of elms in forest restoration? New For. 50, 183–215. https://doi.org/10.1007/s11056-018-9640-x.

Matisone, I., Kenigsvalde, K., Zaļuma, A., Burņeviča, N., Šņepste, I., Matisons, R., Gaitnieks, T., 2020. First report on the Dutch elm disease pathogen *Ophiostoma novo-ulmi* from Latvia. For. Pathol. e12601. https://doi.org/10.1111/efp.12601.

Menkis, A., Östbrant, I.L., Davydenko, K., Bakys, R., Balalaikins, M., Vasaitis, R., 2016. *Scolytus multistriatus* associated with Dutch elm disease on the island of Gotland: phenology and communities of vectored fungi. Mycol. Prog. 15, 1–8. https://doi.org/10.1007/s11557-016-1199-3.

Mittempergher, L., Fagnani, A., Ferrini, F., 1993. An interesting poorly-known elm. The European white elm (*Ulmus laevis* Pallas). Monti-e-Boschi (Italy) 44, 13–17.

Miyashira, C.H., Taniguchi, D.G., Gugliotta, A.M., Santos, D.Y.A.C., 2010. Comparison of radial growth rate of the mutualistic fungus of *Atta sexdens rubropilosa* forel in two culture media. Braz. J. Microbiol. 41, 506–511. https://doi.org/10.1590/S1517-83822010000200035.

Moschenikova, N.B., Vjaznikova, E.V., 2016. Methodological manual for study of Dutch elm disease (Metodicheskoye posobiye po izucheniyu gollandskoy bolezni vyazov) [In Russian].

Motiejūnaitė, J., Kutorga, E., Kasparavičius, J., Lygis, V., Norkutė, G., 2016. New records from Lithuania of fungi alien to Europe. Mycotaxon 131, 49–60. https://doi.org/10.5248/131.49.

Müller, M.M., Hantula, J., Wingfield, M., Drenkhan, R., 2019. *Diplodia sapinea* found on Scots pine in Finland. For. Pathol. 49, e12483. https://doi.org/10.1111/efp.12483.

Mullett, M.S., Adamson, K., Bragança, H., Bulgakov, T.S., Georgieva, M., Henriques, J., Jürisoo, L., Laas, M., Drenkhan, R., 2018. New country and regional records of the pine needle blight pathogens *Lecanosticta acicola*, *Dothistroma septosporum* and *Dothistroma pini*. For. Pathol. 48, e12440. https://doi.org/10.1111/efp.12440.

Mullett, M.S., Drenkhan, R., Adamson, K., Boroń, P., Lenart-Boroń, A., Barnes, I., Tomsovský, M., Jánošíková, Z., Adamčíková, K., Ondrušková, E., Queloz, V., Piškur, B., Musolin, D.L., Davydenko, K., Georgieva, M., Schmitz, S., Kacergius, A., Ghelardini, L., Kranjec Orlović, J., Müller, M., Oskay, F., Hauptman, T., Halász, Á., Markovskaja, S., Solheim, H., Vuorinen, M., Heinzelmann, R., Hamelin, R.C., Konečný, A., 2021. Worldwide genetic structure elucidates the Eurasian origin and invasion pathways of Dothistroma septosporum, causal agent of Dothistroma needle blight. J. Fungi 7, 111. https://doi.org/10.3390/jof7020111.

Napierała-Filipiak, A., Filipiak, M., Łakomy, P., Kuźmiński, R., Gubański, J., 2016. Changes in elm (Ulmus) populations of mid-western Poland during the past 35 years. Dendrobiology 76, 145–156. https://doi.org/10.12657/denbio.076.014.

Nilsson, K., Åkerlund, U., Konijnendijk, C.C., Alekseev, A., Caspersen, O.H., Guldager, S., Kuznetsov, E., Mezenko, A., Selikhovkin, A., 2007. Implementing urban greening aid projects – the case of St. Petersburg, Russia. Urban For. Urban Green. 6, 93–101. https://doi.org/10.1016/J.UFUG.2007.01.004.

Obert, I.S., 1876. List of beetle species collected up to now in Petersburg and its environs (Spisok zhukov najdennih po sie vremia v Peterburge i ego okrestnostiah) [In Russian]. Proc. Russ. Entomol. Soc. Saint Petersbg. (Trudi Rus. Entomol. Obs. v Sankt-Peterburge) 8, 108–139.

Pecori, F., Ghelardini, L., Luchi, N., PEPORI, A.L., Santini, A., 2017. Lights and Shadows of a Possible Strategy to Cope with Alien and Destructive Forest Pathogens: the Example of Breeding for Resistance to Dutch Elm Disease in Italy. Balt. For. 23, 255–263.

Pitney Bowes Software, 2015. Mapinfo https://www.pitneybowes.com/.

R Core Team, 2019. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Rosenvald, R., Drenkhan, R., Riit, T., Lõhmus, A., 2015. Towards silvicultural mitigation of the European ash (*Fraxinus excelsior*) dieback: the importance of acclimated trees in retention forestry. Can. J. For. Res. 45, 1206–1214. https://doi.org/10.1139/cjfr-2014-0512.

Sacchetti, P., Tiberi, R., Mittempergher, L., 1990. Preference of *Scolytus multistriatus* (Marsham) during the gonad maturation phase between two species of elm. Redia 73, 347–354.

Santini, A., Faccoli, M., 2013. Dutch Elm Disease and Elm Bark Beetles: a Century of Association, 8, p. 126. http://iforest.sisef.org/.

Santini, A., Fagnani, A., Ferrini, F., Ghelardini, L., Mittempergher, L., 2005a. Variation among Italian and French elm clones in their response to *Ophiostoma novo-ulmi* inoculation. For. Pathol. 35, 183–193. https://doi.org/10.1111/j.1439-0329.2005.00401.x.

Santini, A., Montaghi, A., Vendramin, G.G., Capretti, P., 2005b. Analysis of the Italian Dutch Elm Disease Fungal Population. J. Phytopathol. 153, 73–79. https://doi.org/10.1111/j.1439-0434.2004.00931.x.

Santini, A., Pecori, F., Pepori, A.L., Ferrini, F., Ghelardini, L., 2010. Genotype × environment interaction and growth stability of several elm clones resistant to Dutch elm disease. For. Ecol. Manage. 260, 1017–1025. https://doi.org/10.1016/J.FORECO.2010.06.025.

Santini, A., Ghelardini, L., De Pace, C., Desprez-Loustau, M.L., Capretti, P., Chandelier, A., Cech, T., Chira, D., Diamandis, S., Gaitniekis, T., Hantula, J., Holdenrieder, O., Jankovsky, L., Jung, T., Jurc, D., Kirisits, T., Kunca, A., Lygis, V., Malecka, M., Marcais, B., Schmitz, S., Schumacher, J., Solheim, H., Solla, A., Szabò, I., Tsopelas, P., Vannini, A., Vettraino, A.M., Webber, J.F., Woodward, S., Stenlid, J., 2013. Biogeographical patterns and determinants of invasion by forest pathogens in Europe. New Phytol. 197, 238–250. https://doi.org/10.1111/j.1469-8137.2012.04364.x.

Selikhovkin, A.V., Popovichev, B.G., Davydova, I.A., Neverovskiy, V.J., 2010. Outbreak of elm bark beetles in St. Petersburg (Massovoye razmnozheniye vyazovykh zabolonnikov v Sankt-Peterburge) [in Russian]. Bull. Int. Acad. Saf. Sci. 14, 5–12.

Selikhovkin, A.V., Popovichev, B.G., Lukmazova, E.A., 2014. The bark beetle *Scolytus pygmaeus* (Fabricius, 1787) in Summer Garden of St. Petersburg (Zabolonnik-pigmey *Scolytus pygmaeus* (Fabricius, 1787) (Coleoptera: Curculionidae: Scolytidae) v Letnem sadu Sankt-Peterburga) [In Russian]. In: Musolin, D.L., Selikhovkin, A.V. (Eds.), Proceedings of the International Conference The Kataev Memorial Readings – VIII. Pests and Diseases of Woody Plants in Russia, Saint Petersburg (Russia). Saint Petersburg State Forest Technical University, Saint Petersburg (Russia), p. 73.

Selikhovkin, A.V., Drenkhan, R., Mandelshtam, M.Y., Musolin, D.L., 2020. Invasions of insect pests and fungal pathogens of woody plants into the northwestern part of European Russia [In Russian]. Vestn. Saint Petersbg. Univ. Earth Sci. 65, 263–283. https://doi.org/10.21638/spbu07.2020.203.

Šerá, B., 2017. Salt-tolerant trees usable for Central European cities-a review. Rev. Hort. Sci. 44, 43–48. https://doi.org/10.17221/201/2015-HORTSCI.

Shcherbakova, L.N., 2008. Elm bark beetles in city green areas of St. Petersburg (Vyazovyye zabolonniki v gorodskikh posadkakh Sankt-Peterburga) [in Russian, Abstract in English]. Izv. Sankt-Peterburgskoj Lesoteh. Akad. (Transactions Saint Petersbg. For. Tech. Acad. 182, 306–313.

Shcherbakova, L.N., Mandelshtam, M.Y., 2014. Elms of Saint Petersburg: after the third ring (Vyazy Sankt Peterburga: posle tret'yego zvonka) [In Russian]. In: Musolin, D. L., Selikhovkin, A.V. (Eds.), Proceedings of the International Conference The Kataev Memorial Readings – VIII. Pests and Diseases of Woody Plants in RuSsia, Saint Petersburg (Russia). Saint Petersburg State Forest Technical University, Saint Petersburg (Russia).

Solheim, H., Eriksen, R., Hietala, A.M., 2011. Dutch elm disease has currently a low incidence on wych elm in Norway. For. Pathol. 41, 182–188. https://doi.org/10.1111/j.1439-0329.2010.00650.x.

Solla, A., Bohnens, J., Collin, E., Diamandis, S., Franke, A., Gil, L., Burón, M., Santini, A., Mittempergher, L., Pinon, J., Vanden Broeck, A., 2005a. Screening European elms for resistance to *Ophiostoma novo-ulmi*. For. Sci. 51, 134–141. https://doi.org/10.1093/forestscience/51.2.134.

Solla, A., Martín, J.A., Corral, P., Gil, L., 2005b. Seasonal changes in wood formation of *Ulmus pumila* and *U. minor* and its relation with Dutch elm disease. New Phytol. 166, 1025–1034. https://doi.org/10.1111/j.1469-8137.2005.01384.x.

St. Petersburg's Parks and Gardens [WWW Document], 2019. URL http://www.saint-petersburn.com/parks/.

Trubacheva, T., Tsymbal, G., Pimenov, K., 2014. Boulevards of the Historical Center of St. Petersburg (Bulvary istoricheskogo tsentra Sankt-Peterburga) [In Russian]. Mikhailovsky Pushkinian 62, 137–145.

Tziros, G.T., Nakopoulou, Z.G., Perlerou, C., Diamandis, S., 2017. Current status of the Dutch elm disease pathogen populations affecting *Ulmus minor* in Greece. For. Pathol. 47, e12323. https://doi.org/10.1111/efp.12323.

Venkova, E., Zanadvorova, V., 1939. The work of the group on the control of pests and diseases of urban greening in 1938 in Leningrad (Rabota gruppy po bor'be s vreditelyami i boleznyami zelenykh nasazhdeniy v 1938g. v Leningrade [In Russian]. Zel. Stroit. (Urban greening) 1–2, 63–66.

Voolma, K., Ounap, H., Süda, I., 2000. Eesti putukate levikuatlas, 2: Ürasklased – Scolytidae (Distribution maps of Estonian insects, 2: Scolytidae) [In Estonian]. Eesti Loodusfoto, Tartu.

Voolma, K., Mandelshtam, M.Y., Shcherbakov, A.N., Yakovlev, E.B., Ounap, H., Süda, I., Popovichev, B.G., Sharapa, T.V., Galasjeva, T.V., Khairetdinov, R.R., Lipatkin, V.A., Mozolevskaya, E.G., 2004. Distribution and spread of bark beetles (Coleoptera: Scolytidae) around the Gulf of Finland: a comparative study with notes on rare species of Estonia, Finland and North-Western Russia. Entomol. Fenn. 15, 198–210.

White, T.J., Bruns, T.D., Lee, S., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J. (Eds.), PCR - Protocols and Applications - A Laboratory Manual. Academic Press, New York, pp. 315–322.

Wingfield, M.J., Brockerhoff, E.G., Wingfield, B.D., Slippers, B., 2015. Planted forest health: the need for a global strategy. Science (80-.) 349, 832–836. https://doi.org/10.1126/science.aac6674.

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Article

Vectors of Dutch Elm Disease in Northern Europe

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Simple Summary: Dutch elm disease (DED) has been killing elms for more than a century in northern Europe; the trees' health status has worsened substantially in recent decades. Elm bark beetles *Scolytus* spp. are vectors of DED. Our aim was to estimate the distribution range of elm bark beetles and to detect potential new vectors of DED agents in northern Europe. Beetles were caught with bottle traps and manually. Then DNA from each specimen was extracted and analysed by the third generation sequencing method. DED agents were detected on the following bark beetles for Europe: *Scolytus scolytus*, S. *triarmatus*, *S. multistriatus*, *S. laevis*, and on new vectors: *Xyleborus dispar* and *Xyleborinus saxesenii*. The spread of *Scolytus triarmatus*, *S. multistriatus* and *Xyleborinus saxesenii* has been remarkable for the last two decades, and *S. triarmatus* and *X. saxesenii* are relatively recent newcomers in the northern Baltics. The problem is that the more vectoring beetles there are, the faster spread of Dutch elm disease from tree to tree.

Abstract: Potential Dutch elm disease vector beetle species were caught with pheromone bottle traps and handpicked in 2019: in total, seven species and 261 specimens were collected. The most common was *Scolytus triarmatus*, but by percent, the incidence of *Ophiostoma novo-ulmi* was highest in *Scolytus scolytus*, followed by *Xyleborinus saxesenii* and *S. triarmatus*. We analysed the beetles' DNA using PacBio sequencing to determine vector beetles of *Ophiostoma novo-ulmi*. *Ophiostoma novo-ulmi* was found on six out of seven analysed beetle species: *Scolytus scolytus*, S. *triarmatus*, *S. multistriatus*, *S. laevis*, *Xyleborinus saxesenii* and *Xyleborus dispar*. The last two beetles were detected as vectors for *Ophiostoma novo-ulmi* for the first time. Previous knowledge on the spread of beetles is discussed.

Keywords: *Scolytus* spp.; DED; *Xyleborinus saxesenii*; *Xyleborus dispar*; pheromone trap; *Ophiostoma novo-ulmi*; climate change; PacBio sequencing

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1. Introduction

There are three native *Ulmus* species (*U. glabra* Huds., *U. laevis* Pall. and *U. minor* Mill.) in northern Europe. *Ulmus glabra,* having more northern range, has spread throughout Estonia; *U. laevis,* having more southern range is growing mainly along riversides; *U. minor* native range reaching at its northernmost extent the Baltic Sea and not to northern Baltics [1,2]. *Ulmus* expanded to Estonia during the Pre-Boreal period, spread rather rapidly, and elms started to decline at the end of the Atlantic period [3].

Elms (*Ulmus* spp.) as a keystone native forest or amenity species have been under attack globally for more than a century due to the Dutch elm disease causal agent *O. ulmi s.l.* [4–8]. Dutch elm disease is a lethal vascular wilt disease, the first symptoms of which are yellowing and browning leaves, a cross-section of an elm twig showing brown spots or streaks in the recent wood rings [9,10].

The first pandemic caused by *O. ulmi* killed 10–40% of elms by 1960 [8,11,12]; the second more severe pandemic killed some 80–90% of mature elms by the beginning of the 21st century in the UK [13,14], as well as hundreds of millions in North America [15]. All native elm species (*U. glabra*, *U. laevis*, *U. minor*) in Sweden endured enormous decimation due to

DED and were registered on the Red List since 2010 as no long-term viable species [16]. DED has been devastating to various *Ulmus* species in Estonia, other Baltic countries and in western Russia since the last decade of the 20th century [17–21]. Ophiostomatoid fungal communities (incl. DED) depend on host trees and vector beetles. Coexistence of fungi and their vectors has been studied [22,23] on conifers [24,25] and considerably less on hardwood species [26].

The mycobiota of xylomycetophagous bark beetles is well studied on ambrosia beetles [27]; less studied in Europe, in particular, are phloem-breeding bark and woodboring beetle-associated fungi; those studies were mainly based on morphological criteria [26]. Bark beetles (Coleoptera: Curculionidae, Scolytinae) are distributed worldwide and form many cosmopolitan genera [28]. Although there are many species of scolytids associated with angiosperm trees (hardwoods) [29], nearly all of them are secondary bark beetles as they colonise stressed or damaged trees [6]. A great majority of scolytid beetles on hardwoods are minor pests and are of no economic importance; an exception—some bark beetles connected with elms [5,29]. Elm bark beetles known to vector DED agents include *Scolytus kirchi* Stalitzky, *S. laevis* Chaupis, *S. multistriatus* Marsham with its variety *triornatus* Eichhoff, *S. pygmaeus* Fabricius, *S. schevyrewi* Semenov, *S. scolytus* Fabricius, *S. triarmatus* Eggers, *S. ulmi* L. Redtenbacher and *Hylurgopinus rufipes* Eichhoff [9,30–34]. Only a few of those are significant [30,35,36], the most important being *Scolytus multistriatus* [4,32,37], *S. scolytus* [30,38] and *S. pygmaeus* [4,36].

Several other species of bark beetles which carry fungal associates in mycangium [39] feed in phloem tissues (inner bark), though some larvae engrave outer sapwood [40], being phloeophagous, preferring mostly species from Ulmaceae [5,9]. Elm bark beetles carry DED pathogen spores on the surface of their body and in their gut [30,36,41,42] from diseased to healthy trees, feeding on and tunnelling in twig crotches of healthy elms, transferring fungal spores to xylem tissues [43]. Fungi spreads inside a branch causing blockage of the conducting system because of the formation of tyloses that cause leaves to wilt and die [32].

Some beetle species that live in association with fungi, e.g., ambrosia beetles are xylomycetophagy—feeding on mycelia, consuming wood incidentally [40]. These are polyphagous species of beetles that inhabit many deciduous tree species, incl. Ulmaceae; those common to northern Europe are *Xyleborus dispar* Fabricius, *Xyleborinus saxesenii* Ratzeburg and *Trypodendron signatum* Fabricius [44–46]. There is a slight possibility that DED could be spread by enthomophagous species that follow bark beetles in their tunnels, e.g., *Salpingus planirostris* and *S. ruficollis*, which are common on deciduous trees in northern Europe. Both had been caught in tunnels of *Scolytus scolytus* in the late stages of development [47]. Similarly, there is a minor possibility that *Paromalus parallelepipedus* and *Hololepta plana* feeding rarely under the bark of elms [48,49] are potential predatory species on *Scolytus* species. Together with elm bark beetles, there are at least seven species of bark beetles in the northern Baltics that can potentially spread the Dutch elm disease agent.

Climate change has caused relatively warmer winters and springs than summers and falls, increasing mean annual temperatures [50–52]. This has an impact on trees in forest ecosystems and in urban areas [53]. For example, *Ulmus glabra* has already started to extend the northern range of its distribution [54,55]. At the same time, climate extremes like unusual fluctuation of temperatures, heavy rains [56], and severe storm events [51] put the hosts under stress and make them susceptible to pests and diseases [51,57]. Those extremities have already caused pathological consequences in Estonian forests [58–63]; new diseases or pests may affect elms, and some may become more aggressive [64,65].

Climate also influences the beetles' outbreaks, their aggressiveness, population dynamics and migration [51] that become more frequent [66]. Usually, the number of scolytid species increased from north to south [67], depending on suitable number of tree species [28]. Warmer climate will probably extend the northern range of *Scolytus* spp. and affect elm trees in the cooler parts of northern Europe [68], as has happened in northwest Russia [52]. As *Scolytus scolytus*, *S. laevis*, *S. multistriatus* are temperature dependent, beetles may be active for a longer period of time than previously, as they start

to fly when the mean temperature is at or above 16 °C [32,33,47] and during an extended warmer period due to climate change.

The aim of the paper is to estimate the distribution of elm bark beetles in Estonia and to detect other potential beetle vectors of DED agents in northern Baltics and the north-western part of Russia.

2. Materials and Methods

2.1. Study Sites and Sampling

The study sites (Figure 1) were chosen among the locations assessed for DED during 2013–2018 [20,21]. All the sampling sites were located in either urban or rural parks; DED had been found in eight park sites and two well-known elm sites were without previous disease conformation. For trapping beetles, species-specific pheromones (semiochemicals) were used [69].

Figure 1. Study sites in Estonia and St. Petersburg, Russia. Bark beetles were handpicked and collected with traps or with both possibilities from the same tree.

Thirty-nine bottle traps (see Figure S1) that contained a 1.5 L plastic bottle with a cut window, plus a smaller bottle with 96% ethanol on the bottom and a pheromone with attractants for *Scolytus* spp. were hung on trees in 10 sites at least 50 m from each other at 3 m above ground as the most effective height [70,71]. Lures consisted of two semipermeable plastic pouches containing a mixture of cubeb oil, 1-hexanol, multistriatin and 4-methyl-3-heptanol (Synergy Semiochemicals Corp., Burnaby, BC, Canada). The lures attract beetles which, while flying towards the lure, hit the plastic sheet, fall into the container with 96% ethanol and sink. We assume that trapped beetles' cross contamination is eliminated in ethanol. Beetles died in ethanol quickly, traps were checked systematically, and the number of beetles was quite low (generally 1–2 individuals and same species per trap). Twenty-three traps were placed in Tallinn, northern Estonia; 16 traps, throughout Estonia (Figure 1).

Traps were hung in the beginning of June 2019 on what were at the time visually healthy elm trees; sampling was carried out from the second half of June until the beginning of September, at least every three weeks, five times total during the season.

Scolytus-like beetles were immersed in 96% ethanol for sterilisation and were put in separate tubes and stored in −20 °C until further processing.

To secure a sufficiently large sample size for DNA analysis, we also hand-picked beetles from bark surface of trees at three sites in Estonia (four trees) and one site in St. Petersburg, Russia (three trees). Each specimen was captured into a separate sterile tube.

2.2. Beetles' Identification

All beetles caught were identified using an Olympus stereo zoom microscope SZ60 (Olympus Corporation, Japan) with 100× maximum magnification, based on the following identification keys [72–76]. If necessary, genitals were separated, and the sex of the bark beetles were determined. There were 319 determined beetles in total.

2.3. Molecular Analyses

DNA was extracted separately from each beetle's whole body [77] using GeneJET Genomic DNA purification kit (Thermo Fischer Scientific, Vilnius, Lithuania). All 259 collected and determined possible vector beetles were DNA extracted from which 109 were randomly selected, covering different beetle species, sex, locations, and sampling for future analyses.

Primers ITS4ngsUni [78] and ITS1catta [79] were used to amplify fungal DNA and the PCR products were sequenced using PacBio sequencing in the University of Oslo in Norway. Both reverse and forward primers were equipped with 109 different MID tags with 10–12 base length (different pair per sample) that had at least 4 base differences from one another. PacBio has recently been successfully used in metabarcoding analysis of microorganisms on various trees and plants, as the long DNA barcodes of 500–1500 bp can improve OTU identification on a species level [80–82].

Conventional PCR was carried out according to [82] with two replicates for each sample in 25 µL reaction volume containing 0.5 µL of forward and reverse primer and 5 µL of HOT FIRE Pol Blend Master Mix Ready to Load (Solis BioDyne, Tartu, Estonia). Amplification was performed as follows: 15 min at 95 °C, followed by 25 cycles of 30 s at 95 °C; 30 s at 55 °C; 1 min at 72 °C, and a final step at 72 °C for 10 min. Positive and negative controls were used throughout the analysis to exclude possible tag switches and sample contamination during the PCR process.

The PCR reactions were checked for the presence of a product on 1% agarose gel. In the case of no visible band, we repeated the amplification by increasing the number of cycles up to 35. The PCR products were purified using FavorPrep™ GEL/PCR Purification Kit (Favorgen, Vienna, Austria) following the manufacturer's instructions.

The amplicons were pooled into one sequencing library. Library preparation followed the protocols established for the RSII instrument of PacBio third-generation sequencing platform (Pacific Biosciences, Inc. Menlo Park, CA, USA). The diffusion method was used in loading the library to SMRT cells. Sequencing was performed using P6-C4 chemistry for 10 h following Tedersoo et al. [83].

2.4. Bioinformatics and Statistical Analysis

Bioinformatics was carried out by using various programs implemented in Pipecraft v1.0 [84].

Using mothur (v1.36.1) [85], reads < 100 bp were removed and longer sequences were demultiplexed allowing 2-base differences to index and 3-base differences to primer. UCHIME [86] was used in de novo chimera filtering. The full-length Internal Transcribed Spacer (ITS) region was extracted from the rRNA genes with program ITSx (v1.0.11) [87]. CD-HIT (v4.6) [87] was used to cluster sequences into Operational Taxonomic Units (OTUs) based on 97% sequence similarity. OTUs were taxonomically identified based on representative sequences against the UNITE v.7 database [88]. OTUs were considered as members of fungi if their representative sequences matched the best fungal taxa at e-value < e−50. Representative sequences that had >97% sequence similarity to reference sequences were assigned to species hypotheses (SHs) based on UNITE [89]. Higher level classification of fungi was based on the e-value and sequence similarity criteria of Tedersoo et al. [78].

Differences of percentage of *O. novo-ulmi* between sampling methods, sampling areas, beetle species and genders were analysed using ANOVA with Tukey HSD in Excel and were considered significant with p value ≤ 0.05.

3. Results

3.1. Collected Beetle Species

In total, 319 specimens of beetles (28 different species) were caught, from which 93 specimens of 23 beetle species were captured with pheromone-baited bottle traps (28 of the 39 traps contained beetles). The number of potential vector beetle individuals for DED was 261, from which 81% of beetles were handpicked in four sites from seven different trees; 9% were trapped. The number of potential DED vector beetles collected with traps and symptomatic trees are presented by species, country, and gender in Table 1. The other beetle species collected are listed in Supplementary Materials Table S1.

Table 1. Potential vector beetle species of DED caught with traps and handpicked from symptomatic trees.

Species of Beetles	Country	Traps		Handpicked		Total
		Sex		Sex		
		Male	Female	Male	Female	
Scolytus multistriatus	Estonia	3	3	-	-	6
	Russia	-	-	5	-	5
Scolytus triarmatus	Estonia	4	2	66	114	186
Scolytus laevis	Estonia	-	-	21	18	39
Scolytus scolytus	Russia	-	-	2	2	4
Scolytus pygmaeus	Russia	-	-	-	1	1
Xyleborinus saxesenii	Estonia	-	10	-	-	10
Xyleborus dispar	Estonia	-	10	-	-	10
	Total	32		229		261

3.2. Ophiostoma novo-ulmi on Vector Beetles

The number of sequenced individuals was 109; the selection was based on beetle specimens that covered different beetle species, sex, locations and different collecting methods.

The entire sequenced dataset consisted of 33,202 high quality sequences across 109 beetle specimen samples and 655 OTUs (see Genbank accession number PRJNA719602).

Ophiostoma novo-ulmi was found on six out of seven beetle species. Only *S. pygmaeus* had no *O. novo-ulmi*. Among the most caught species was *S. triarmatus;* the pathogen was detected in 77% of analysed specimens.

In total, 2757 *O. novo-ulmi* ITS sequences were found in the dataset, constituting 8.3% of all sequences within the dataset (see https://dx.doi.org/10.15156/BIO/807454, accessed on 15 April 2021). *Ophiostoma novo-ulmi* was the most prevalent species in this dataset. The highest average percentage of *O. novo-ulmi* per sample was found on beetle species *S. scolytus,* followed by *X. saxesenii* and *S. triarmatus* with 26.6%, 20.5% and 18.2%, respectively; although considerable, these differences among species were not statistically significant, possibly due to a large variation in sample sizes among species ($F_{5,101} = 1.89$; $p = 0.101$; Figure 2). The difference between genders was also not statistically significant ($F_{5,101} = 0.001$; $p = 0.993$). Comparison between handpicked and trapped specimens resulted no significant difference in percentages of *O. novo-ulmi* ($F_{1,80} = 0.04$; $p = 0.848$). The beetles handpicked directly from trees had 8.7% of sequences identified as *O. novo-ulmi,* whereas 8.1% of *O. novo-ulmi* was found on beetles collected with traps. When comparing the relative abundance of *O. novo-ulmi* across 15 different sampling sites and all beetle species, the highest percentage of *O. novo-ulmi* was found in site Tallinn (Kopli), North Estonia, followed by site Vastseliina, Southeast Estonia and site St. Petersburg, Russia with 27.7%, 18.7% and 15.4%, respectively. According to ANOVA with Tukey HSD *O. novo-ulmi*

percentage differences between sites were not statistically significant ($F_{8,91} = 1.42$; $p = 0.196$). For more precise distribution data of *O. novo-ulmi* across the sampling sites, beetle species and gender, see Supplementary Table S2.

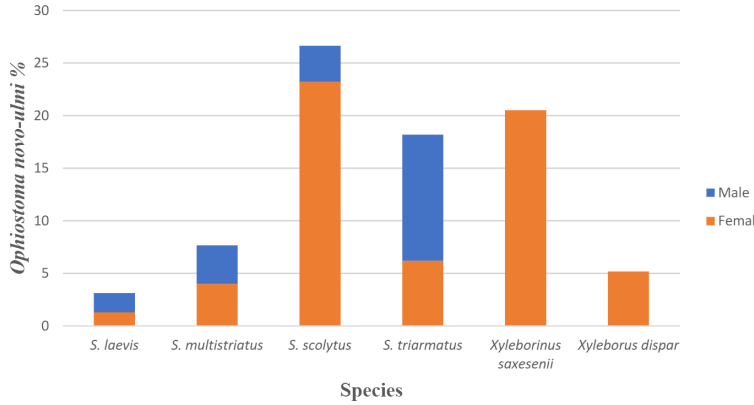

Figure 2. Percentage of *O. novo-ulmi* across different beetle species and gender (N = 109). No *O. novo-ulmi* was found on *S. pygmaeus*.

4. Discussion

4.1. Beetles as Vectors of O. novo-ulmi and Pathogen Detection

When referring to beetles that spread Dutch elm disease, in particular, we mean those species that can inhabit living elms, their bark and/or wood.

According to PacBio sequencing, we found the pathogen in six commonly captured beetle species (*S. scolytus*, *S. laevis*, *S. multisriatus*, *S. triarmatus*, *X. saxesenii*, *X. dispar*; see Figure 2). Among those, *X. saxesenii* and *X. dispar* were found as new vectors for Dutch elm disease in northern Europe. *Ophiostoma novo-ulmi* was not detected on *S. pygmaeus* in this study.

Bark and ambrosia beetles are an optimal vehicle for the transport of different organisms, incl. fungi, from one host to another [40].

Until now, the main culprits in the spread of Dutch elm disease have been *Scolytus* spp. [4,32,37]. Knowing the biology of Scolytinae and their suitable host trees, the range of these potential vector species is somewhat wider. In addition to *Scolytus* sp., host species from family Ulmaceae are inhabited by at least the following species of beetles common in northern Europe: *Xyleborus dispar*, *Xyleborinus saxesenii* and *Trypodendron signatum* [44–46]. These are polyphagous pests that inhabit many deciduous tree species but may rarely occur in conifers as well. *Xyleborus dispar* can also attack healthy trees, especially when those are close to stressed hosts [89,90].

Most ambrosia beetles do not feed on wood, but *Xyleborinus saxesenii* is an exception. The larvae feed on the ectosymbiotic fungus growing in wood and the wood itself, classifying this species as xylomycetophagous, rather than just mycetophagous (feeding only on fungi) [91]. *Xyleborinus saxesenii* is strongly attracted to ethanol-based baits and often accumulates in ethanol-baited traps in numbers greater than other ambrosia beetles [92]. Most cases where the species is reported as aggressive to stressed hosts include elms [93].

In Poland *O. novo-ulmi* subsp. *novo-ulmi* was isolated not from only the elm-infecting beetles but also from *Hylesinus crenatus* on *Fraxinus excelsior* and *Scolytus intricatus* on

Quercus robur [22]. *Ophiostoma novo-ulmi* was found on an unknown vector and on other host species than elms, thus the pathogen occurrence in forest ecosystems is much broader than previously thought [22,94].

The pathogen detection from biological samples incl. vector beetles is highly crucial to estimate the disease spread and occurrence. Thus, we note that primers ITS1catta and ITS4ngsUni and PacBio sequencing platform worked well identifying *O. novo-ulmi* from different bark beetle species. The primers were able to distinguish *O. novo-ulmi* across 33,202 ITS sequences and 655 OTU-s. *Ophiostoma novo-ulmi* was the most prevalent species in this dataset: 8.3% of sequences were identified as *O. novo-ulmi* with the average length of *O. novo-ulmi* amplicons being 612 bp. We sequenced the ITS region, which does not differentiate *O. novo-ulmi* subspecies, but we have evidence that the subspecies may have different aggressiveness (see [17,95]). Thus, species-specific DNA primers are needed to differentiate *O. novo-ulmi* subspecies from biological samples for faster detection of the spread of pathogen.

4.2. Spread of Vectors for DED in Estonia and Northwest Russia

Scolytus bark beetles are the main vectors for the transmission of DED, introducing the pathogen into visually healthy trees during adult feeding and breeding.

Scolytus multistriatus was found to vector *O. novo-ulmi* in the current work and the pathogen was detected in about 8% of analysed individuals. The northernmost European finding of *Scolytus multistriatus* was recorded in a park in St. Petersburg in 1997 [96,97], and later in Vyborg (B.G. Popovichev, pers. comm.). It was first registered in Estonia, close to Tartu in about 1900 [98], then was rediscovered in southern Estonia: Taheva (1967) and Karisöödi (1996) [99]. Since 2012, the species has been relatively abundant on the Koiva wooded meadow near Vaitka, South Estonia. According to the latest data, in 2019, *S. multistriatus* has already reached central Estonia.

Scolytus laevis, of which 2.5% of the analysed individuals contained the pathogen, is the most widely spread species of its genera in Estonia and Russia [100] but has been found much farther north in Sweden and Norway than in the central parts of the Leningrad Region [96,97]. It was recorded for the first time in South Estonia at Heimtali, Viljandi County in 1936 [101], a few years later, in 1938, also from Viljandi, central Estonia [102]. *Scolytus laevis* was also found in northern Estonia [103] and is now widespread presumably throughout the Estonian mainland. Years ago, DED was rarely spread by this species [100] but it has been currently proved as a vector of DED in our region.

Scolytus scolytus, of which more than fourth of analysed specimens contained *O. novo-ulmi*. The northernmost recordings of *S. scolytus* in Europe were found in St. Petersburg's city parks in 2000 [97,104] and later in Vyborg (B.G. Popovichev, pers. comm.). It should be mentioned that in Sweden, *S. scolytus* has been completely replaced by the related species *S. triarmatus*, which was reported from even farther northern Sweden, compared with *S. scolytus* in Estonia [104]. Only two dead specimens were found in Estonia on the island of Abruka in western Estonia (leg. et det. I. Süda): 1 ♀under the bark of on old dead elm in 1994 and 1 ♂under the bark of a thick branch of an old elm in 1997 [99,105,106].

Scolytus triarmatus, of which 18% of analysed specimens carried *O. novo-ulmi.* The first finding of *S. triarmatus* in Estonia (also in the Baltics) originated from Soontaga, southern Estonia in 2004 [106]. It is noteworthy that in 2012, in the park of Linnamäe manor in south-eastern Estonia, the entire trunk of one of Estonia's thickest elms (CBH = 5 m) was quite massively inhabited by this species (observation by I. Süda). *S. triarmatus* has now strongly expanded its distribution in Estonia. In 2019, it was caught from Tartu, Vastseliina, Viljandi, Hummuli in southern Estonia and several sites in Tallinn, northern Estonia. In addition, all the above-mentioned vectors of DED occur in the northern European part of Russia as well, except *S. triarmatus* [75].

Scolytus pygmaeus has not been detected in Estonia as of yet, but recently appeared in northwest Russia in 2012 [97,100,107], being native in the central Russian territories [108]. It is likely to have spread along the roadside of the highway from Moscow to St. Peters-

burg [97], where planted elm stands served as corridors for leading northwards [109]; the same is true of *S. multistriatus* and *S. scolytus.* One single individual was collected from Russia, but *O. novo-ulmi* was not detected. *Scolytus pygmaeus* was found to be the vector of pathogen [110].

Ophiostomatoid fungi associate with phloem-breeding and ambrosia beetles on hardwoods [22] but there are some notes indicating higher species diversity as vectors than that previously reported from Europe [22].

Xyleborinus saxesenii—20% of analysed specimens contained the pathogen. The first record of *X. saxesenii* was in western Estonia from the Laulaste Nature Reserve, southeastern Estonia in 2008 and from Matsalu, western Estonia in 2009 [111]. Later, *X. saxesenii* was found from two localities in South Estonia, Valga County: Koiva wooded meadow in 2013 and Soontaga in 2015, 2020. This work confirmed the beetle's first finding in 2019 and it was caught with traps from several localities in Tallinn, northern Estonia. *Xyleborinus saxesenii* is capable of breeding in various hosts [24,93] including elms [112]. Like other ambrosia beetles, *X. saxesenii* breeds mostly in weakened or dying trees. *Xyleborinus saxesenii* has been considered an insignificant pest until recently, when it has been proved to spread *O. novo-ulmi*. Additionally, *X. saxesenii* has been shown to be able to spread the laurel wilt pathogen *Raffaelea lauricola* (Ophiostomatales) [14,112,113].

Xyleborus dispar and *Trypodendron signatum* are known to occur on a wide range of deciduous trees [113,114]. Both species are native, common and widespread throughout Estonia, but the latter was not found on elms. However, *Xyleborus dispar* was proved to be the vector of *O. novo-ulmi* in this work, e.g., 5% of analysed specimens contained *O. novo-ulmi*.

The implementation of such highly efficient research methods as the use of window traps has helped to detect new woodland beetle species for Estonian fauna [111,115]. On the other hand, as a result of climate change, the spread of numerous southern beetle species to the north is clearly noticeable [115], especially in the last couple of decades. The same is true for the DED vectors *Scolytus multistriatus* and *Xyleborinus saxesenii.* However, it is not clear why *Scolytus triarmatus* has become widespread and numerous in Estonia in such a short time. Considering that *Scolytus triarmatus* does not occur in Latvia, Lithuania nor Finland, the ambiguity is even greater.

4.3. The Traps and Alcohol as a Baiting Compound

Bark beetles are strongly attracted to synthetic pheromone [71,116] thus pheromone traps can be used to indicate their presence.

Traps used in our research were made from 1.5 L plastic bottles, because these are cost effective [92]. The number of species and specimens caught with pheromone-baited bottle traps used in this study indicate quite low efficiency as used in current work. If the pheromone was created to attract specifically for *Scolytus* spp., then ethanol is known to affect many saproxylic beetles, including bark beetles [45,117]. A comparative study in France proved that the overall specimen collecting efficiency of alcohol-baited traps in catching saproxylic beetles was twice as large as that of nonbaited traps, the efficiency ratio amounting even to 114 in catching *Xyleborinus saxesenii*! [117]. It can be assumed that in Estonia too, a relatively large number of *Xyleborinus saxesenii* and *Xyleborus dispar* specimens were lured into pheromone traps due to the attractiveness of ethanol; this does not directly indicate the high abundance of both species in the study sites. It is probable that ethanol has also an attracting effect on *Scolytus* spp. species. During the sampling, there were usually 1–2 specimens per trap at a time, so cross-contamination was diminished between same species individuals and different species. Thus, low efficiency of trapping fit well with the current work tasks.

Possible vectors could be also predators of Scolytinae: *Salpingus planirostris* and *Paromalus parallelepipedus* [118]. *Salpingus planirostris* is a common species mainly found on deciduous trees including *Ulmus*, and *P. parallelepipedus* inhabits mainly conifers, occasion-

ally some deciduous trees as well, including *Ulmus* [49]. Therefore, it is not a coincidence that some specimens were caught on elms in Estonia.

5. Conclusions

This study provides new information on vectors for Dutch elm disease in northern Europe. From all 319 beetle specimens caught either with traps or handpicked—261 specimens were potential vectors of Dutch elm disease. High throughput sequencing indicated that *Ophiostoma novo-ulmi* was the most represented fungus (8.2%) on six out of seven analysed beetle species. The highest percentage of *O. novo-ulmi* was found on beetle species *S. scolytus,* followed by *X. saxesenii* and *S. triarmatus.*

According to the latest studies of potential DED vectors, the rather rapid expansion of the distribution of three species—*Scolytus triarmatus*, *S. multistriatus* and *Xyleborinus saxesenii*—in Estonia is remarkable. The first two have expanded northwards, currently spreading to the centre line of Estonia, *S. triarmatus* in addition to north-western Estonia. It is noteworthy that *S. triarmatus* and *X. saxesenii* are relatively recent newcomers in Estonia, first registered in 2004 and 2008, respectively. Whereas previously the most common *Scolytus*-species on elms in Estonia was *S. laevis*, now it seems to be *S. triarmatus*.

In conclusion, the results of our work with known elm bark beetles (*Scolytus laevis*, *S. multistriatus*, *S. triarmatus* and *S. scolytus*), indicates that there are two more species that can potentially spread Dutch elm disease in Estonia: *Xyleborus dispar* and *Xyleborinus saxesenii*. We did not catch one potential DED vector *Trypodendron signatum* on *Ulmus,* thus a connection with *O. novo-ulmi* cannot be confirmed in Estonia.

Since this was a monitoring study, there is not enough data on the extent of infection in the areas—differences between sampling sites, beetle species and sexes. A study of these named aspects could be the focus of future work.

The spread of DED should be controlled with ongoing survey of trees and the monitoring of vectoring beetles with the help of pheromone traps. Removing of all diseased elms as soon as possible is a key factor to protect healthy elms. Additionally, new and reliable species-specific DNA primers are needed for quick pathogen detection from biological samples to control disease spread.

Supplementary Materials: The following are available online at https://dx.doi.org/10.15156/BIO/807454, Figure S1: In current work used bottle trap with pheromone; Table S1: Other insect species caught with traps and handpicked; Table S2: Relative abundance of *O. novo-ulmi* across different sampling sites and beetle species.

Author Contributions: The original idea and study design were developed by L.J., R.D. and I.S. Data and samples collection was provided by L.J. and I.S. Laboratory work, data analysis, and interpretation of results were provided by L.J., A.A. and I.S. The first draft of the manuscript was written by L.J. and A.A. The statistical analyses were chosen and overseen by A.A. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: Full ITS sequences of the entire dataset are uploaded into SRA under accession number PRJNA719602. Filtered representative full ITS sequences of *O. novo-ulmi* are uploaded into PlutoF: https://dx.doi.org/10.15156/BIO/807454, accessed on 15 April 2021.

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References

1. Kukk, T.; Kull, T. *Eesti Taimede Levikuatlas (Atlas of the Estonian Flora)*; Eesti Maaülikooli Põllumajandus- ja Keskkonnainstituut; Institute of Agricultural and Environmental Sciences of the Estonian University of Life Sciences: Tartu, Estonia, 2005; 528p. (In Estonian)
2. Caudullo, G.; De Rigo, D. *Ulmus*—elms in Europe: Distribution, habitat, usage and threats. In *European Atlas of Forest Tree Species*; San-Miguel-Ayanz, J., de Rigo, D., Caudullo, G., Durrant, H.T., Mauri, A., Eds.; Publications Office of the EU: Luxembourg, 2016; p. e01bd40.
3. Saarse, L.; Veski, S. Spread of broad-leaved trees in Estonia. *Proc. Est. Acad. Sci. Geol.* **2001**, *50*, 51. Available online: https://www.researchgate.net/publication/252516467_Spread_of_broad-leaved_trees_in_Estonia (accessed on 6 April 2021).
4. Santini, A.; Faccoli, M. Dutch elm disease and elm bark beetles: A century of association. *iForest Biogeosci. For.* **2015**, *8*, 126–134. [CrossRef]
5. Smith, S.M.; Hulcr, J. *Scolytus* and other Economically Important Bark and Ambrosia Beetles. *Bark Beetles* **2015**, 495–531. [CrossRef]
6. Wingfield, M.J.; Garnas, J.R.; Hajek, A.; Hurley, B.P.; De Beer, Z.W.; Taerum, S.J. Novel and co-evolved associations between insects and microorganisms as drivers of forest pestilence. *Biol. Invasions* **2016**, *18*, 1045–1056. [CrossRef]
7. Martín, J.A.; Sobrino-Plata, J.; Rodríguez-Calcerrada, J.; Collada, C.; Gil, L. Breeding and scientific advances in the fight against Dutch elm disease: Will they allow the use of elms in forest restoration? *New For.* **2019**, *50*, 183–215. [CrossRef]
8. Brasier, C.M. Intercontinental Spread and Continuing Evolution of the Dutch Elm Disease Pathogens. In *The Elms*; Springer Science and Business Media LLC: Berlin/Heidelberg, Germany, 2000; pp. 61–72.
9. Clinton, G.P.; McCormick, F.A. *Dutch Elm Disease. Graphium Ulmi*; Connecticut Agricultural Experimental Station, Bulletin 386: New Haven, CT, USA, 1936; pp. 701–752.
10. Stipes, R.J.; Campana, R.J. *Compendium of Elm Diseases*; Stipes, R.J., Campana, R.J., Eds.; American Phytopathological Society: Saint Paul, MN, USA, 1981.
11. Peace, T.R. The status and development of Elm Disease in Britain. *Comm Bull.* **1960**, *33*, 44.
12. Gibbs, J.N. Intercontinental Epidemiology of Dutch Elm Disease. *Annu. Rev. Phytopathol.* **1978**, *16*, 287–307. [CrossRef]
13. Brasier, C.; Buck, K. Rapid Evolutionary Changes in a Globally Invading Fungal Pathogen (Dutch Elm Disease). *Biol. Invasions* **2001**, *3*, 223–233. [CrossRef]
14. Kirisits, T. Dutch elm disease and other *Ophiostoma* diseases. *Infect. For. Dis.* **2013**, *2013*, 256–282. [CrossRef]
15. Brasier, C.M. Rapid Evolution of Introduced Plant Pathogens via Interspecific Hybridization is leading to rapid evolution of Dutch elm disease and other fungal plant pathogens. *Bioscience* **2001**, *51*, 123–133. [CrossRef]
16. Gärdenfors, U. The 2010 Red List of Swedish Species. Available online: https://www.artdatabanken.se/globalassets/ew/subw/artd/2.-var-verksamhet/publikationer/4.-rodlista-2010/274614_inlaga_liten_sid-del1-1-199.pdf (accessed on 27 November 2019).
17. Jürisoo, L.; Selikhovkin, A.V.; Padari, A.; Shevchenko, S.V.; Shcherbakova, L.N.; Popovichev, B.G.; Drenkhan, R. The extensive damages of elms by Dutch elm disease agents and their hybrids in north-western Russia. *Urban For. Urban Green.* **2021**. submitted.
18. Matisone, I.; Kenigsvalde, K.; Zaļuma, A.; Burņeviča, N.; Šņepste, I.; Matisons, R.; Gaitnieks, T. First report on the Dutch elm disease pathogen *Ophiostoma novo-ulmi* from Latvia. *For. Pathol.* **2020**, e12601. [CrossRef]
19. Motiejūnaitė, J.; Kutorga, E.; Kasparavičius, J.; Lygis, V.; Norkutė, G.; Jurga, M.; Ernestas, K.; Jonas, K.; Vaidotas, L.; Goda, N. New records from Lithuania of fungi alien to Europe. *Mycotaxon* **2016**, *131*, 49–60. [CrossRef]
20. Jürisoo, L.; Padari, A.; Drenkhan, R. Jalakasurma levikust ja ohtlikkusest Eestis (Spread and riskiness of Dutch elm disease in Estonia). *For. Stud. Metsanduslikud Uurim.* **2021**, submitted. (In Estonian)
21. Jürisoo, L.; Adamson, K.; Padari, A.; Drenkhan, R. Health of elms and Dutch elm disease in Estonia. *Eur. J. Plant Pathol.* **2019**, *154*, 823–841. [CrossRef]
22. Jankowiak, R.; Strzałka, B.; Bilański, P.; Kacprzyk, M.; Wieczorek, P.; Linnakoski, R. Ophiostomatoid fungi associated with hardwood-infesting bark and ambrosia beetles in Poland: Taxonomic diversity and vector specificity. *Fungal Ecol.* **2019**, *39*, 152–167. [CrossRef]
23. Pajares, J.A.; García, S.; Díez, J.J.; Martín, D.; García-Vallejo, M.C. Feeding Responses by *Scolytus scolytus* to Twig Bark Extracts from Elms. *Investig. Agraria Sist. Recur. For.* **2004**, *13*, 217–225.
24. Kirisits, T. Fungal Associates of European Bark Beetles with Special Emphasis on the Ophiostomatoid Fungi. In *Bark and Wood Boring Insects in Living Trees in Europe, a Synthesis*; Metzler, J.B., Ed.; Springer: Berlin, Germany, 2007; Volume 2007, pp. 181–236.
25. Linnakoski, R. Bark beetle-associated fungi in Fennoscandia with special emphasis on species of *Ophiostoma* and *Grosmannia*. *Diss. For.* **2011**, *2011*. [CrossRef]
26. Aas, T.; Solheim, H.; Jankowiak, R.; Bilański, P.; Hausner, G. Four new *Ophiostoma* species associated with hardwood-infesting bark beetles in Norway and Poland. *Fungal Biol.* **2018**, *122*, 1142–1158. [CrossRef]
27. Mayers, C.G.; McNew, D.L.; Harrington, T.C.; Roeper, R.A.; Fraedrich, S.W.; Biedermann, P.H.; Castrillo, L.A.; Reed, S.E. Three genera in the Ceratocystidaceae are the respective symbionts of three independent lineages of ambrosia beetles with large, complex mycangia. *Fungal Biol.* **2015**, *119*, 1075–1092. [CrossRef]
28. Heliövaara, K.; Peltonen, M. Bark Beetles in a Changing Environment. *Ecol. Bull.* **1999**, 48–53. [CrossRef]
29. Ohmart, C.P. Why are There so Few Tree-Killing Bark Beetles Associated with Angiosperms? *Oikos* **1989**, *54*, 242. [CrossRef]

30. Webber, J.F. Relative effectiveness of *Scolytus scolytus*, *S. multistriatus* and *S. kirschi* as vectors of Dutch elm disease. *For. Pathol.* **1990**, *20*, 184–192. [CrossRef]
31. Sherif, S.; Jones, A.; Shukla, M.; Saxena, P. Establishment of invasive and non-invasive reporter systems to investigate American elm–*Ophiostoma novo-ulmi* interactions. *Fungal Genet. Biol.* **2014**, *71*, 32–41. [CrossRef]
32. Menkis, A.; Östbrant, I.-L.; Davydenko, K.; Bakys, R.; Balalaikins, M.; Vasaitis, R. *Scolytus multistriatus* associated with Dutch elm disease on the island of Gotland: Phenology and communities of vectored fungi. *Mycol. Prog.* **2016**, *15*, 1–8. [CrossRef]
33. Anderbrant, O.; Schlyter, F. Ecology of the Dutch Elm Disease Vectors *Scolytus laevis* and *S. scolytus* (Coleoptera: Scolytidae) in Southern Sweden. *J. Appl. Ecol.* **1987**, *24*, 539. [CrossRef]
34. Anderbrant, O.; Yuvaraj, J.K.; Martin, J.A.; Rodriguez-Gil, J.L.; Witzell, J. Feeding by *Scolytus* bark beetles to test for differently susceptible elm varieties. *J. Appl. Entomol.* **2017**, *141*, 417–420. [CrossRef]
35. Tarigan, M.; Roux, J.; Van Wyk, M.; Tjahjono, B.; Wingfield, M. A new wilt and die-back disease of *Acacia mangium* associated with *Ceratocystis manginecans* and *C. acaciivora* sp. nov. in Indonesia. *South. Afr. J. Bot.* **2011**, *77*, 292–304. [CrossRef]
36. Webber, J.F. Experimental studies on factors influencing the transmission of Dutch elm disease. *Investig. Agrar. Sist. Recur.* **2004**, *13*, 197.
37. Lindelöw, Å. Introduced or Overlooked? New Bark Beetle Species in Sweden (Coleoptera; Curculionidae). *Forstsch. Aktuell.* **2012**, *55*, 28. Available online: https://bfw.ac.at/400/pdf/fsaktuell_55_10.pdf (accessed on 24 May 2019).
38. Waller, M. Drought, disease, defoliation and death: Forest pathogens as agents of past vegetation change. *J. Quat. Sci.* **2013**, *28*, 336–342. [CrossRef]
39. Hulcr, J.; Stelinski, L.L. The Ambrosia Symbiosis: From Evolutionary Ecology to Practical Management. *Annu. Rev. Entomol.* **2017**, *62*, 285–303. [CrossRef] [PubMed]
40. Kirkendall, L.R.; Biedermann, P.H.; Jordal, B.H. Evolution and Diversity of Bark and Ambrosia Beetles. *Bark Beetles* **2015**, *2015*, 85–156. [CrossRef]
41. Bernier, L.; Aoun, M.; Bouvet, G.; Comeau, A.; Dufour, J.; Naruzawa, E.; Nigg, M.; Plourde, K. Genomics of the Dutch elm disease pathosystem: Are we there yet? *iForest Biogeosci. For.* **2015**, *8*, 149–157. [CrossRef]
42. Moser, J.C.; Konrad, H.; Blomquist, S.R.; Kirisits, T. Do mites phoretic on elm bark beetles contribute to the transmission of Dutch elm disease? *Naturwissenschaften* **2009**, *97*, 219–227. [CrossRef] [PubMed]
43. Sherif, S.M.; Erland, L.A.; Shukla, M.R.; Saxena, P.K. Bark and wood tissues of American elm exhibit distinct responses to Dutch elm disease. *Sci. Rep.* **2017**, *7*, 1–11. [CrossRef]
44. Koch, K. *Die Kaefer Mitteleuropas*, 3rd ed.; Goecke & Evers: Krefeld, Germany, 1992; 370p.
45. Nikulina, T.; Mandelshtam, M.; Petrov, A.; Nazarenko, V.; Yunakov, N. A survey of the weevils of Ukraine. Bark and ambrosia beetles (Coleoptera: Curculionidae: Platypodinae and Scolytinae). *Zootaxa* **2015**, *3912*, 1–61. [CrossRef]
46. Yanovskiy, V.M. Annotirovannyy spisok koroyedov (Scolytidae) Severnoy Azii (Annotated list of bark beetles (Scolytidae) of North Asia). *Entomol. Rev.* **1999**, *78*, 327–362. (In Russian)
47. Beaver, R.A. Notes on the fauna associated with elm bark beetles in Wytham Wood, Berks—I Coleoptera. *Entomol. Mon. Mag.* **1966**, *102*, 163–170.
48. Borowski, J.; Mazur, S. Beetles (Coleoptera) of the Rogów Region—Part IV—Clown Beetles (Histeridae) and False Clown Beetles (Sphaeritidae). *Int. Lett. Nat. Sci.* **2015**, *37*, 10–17. [CrossRef]
49. Troukens, W. *Paromalus parallelepipedus* (Coleoptera: Histeridae) aan de westrand van Brussel (*Paromalus parallelepipedus* (Coleoptera: Histeridae) at the westside of Brussels). *Phegea* **2015**, *43*. Available online: www.faunaeur.org (accessed on 21 January 2021). (In Dutch).
50. Vose, R.S.; Easterling, D.R.; Gleason, B. Maximum and minimum temperature trends for the globe: An update through *Geophys. Res. Lett.* **2005**, *32*, 1–5. [CrossRef]
51. Bentz, B.J.; Jönsson, A.M. Modeling Bark Beetle Responses to Climate Change. *Bark Beetles* **2015**, *2015*, 533–553. [CrossRef]
52. Selikhovkin, A.V.; Drenkhan, R.; Mandelshtam, M.Y.; Musolin, D.L. Invasions of insect pests and fungal pathogens of woody plants into the northwestern part of European Russia. *Vestnik St. Petersburg Univ. Earth Sci.* **2020**, *65*, 263–283. [CrossRef]
53. Sturrock, R.N.; Frankel, S.J.; Brown, A.V.; Hennon, P.E.; Kliejunas, J.T.; Lewis, K.J.; Worrall, J.J.; Woods, A.J. Climate change and forest diseases. *Plant Pathol.* **2011**, *60*, 133–149. [CrossRef]
54. Kullman, L. Changes in alpine plant cover—effects of climate warming. *Sven. Bot. Tidskr.* **2003**, *97*, 210.
55. Drobyshev, I.V. Effect of natural disturbances on the abundance of Norway spruce (*Picea abies* (L.) Karst.) regeneration in nemoral forests of the southern boreal zone. *For. Ecol. Manag.* **2001**, *140*, 151–161. [CrossRef]
56. Roloff, A.; Korn, S.; Gillner, S. The Climate-Species-Matrix to select tree species for urban habitats considering climate change. *Urban For. Urban Green.* **2009**, *8*, 295–308. [CrossRef]
57. Hanso, M.; Drenkhan, R. Metsa-ja linnapuud ilmastiku äärmuste vaevas (Trees in forests and towns are suffering from the extreme weather conditions). *Eesti Lood Est. Nat.* **2007**, *58*, 6–13. (In Estonian)
58. Hanso, M.; Drenkhan, R. Simple visualization of climate change for improving the public perception in forest pathology/Kliimamuutuste visualiseerimise lihtne viis seoste paremaks tajumiseks metsapatoloogias. *For. Stud.* **2013**, *58*, 37–45. [CrossRef]
59. Adamson, K.; Drenkhan, R.; Hanso, M. Invasive brown spot needle blight caused by *Lecanosticta acicola* in Estonia. *Scand. J. For. Res.* **2015**, *30*, 587–593. [CrossRef]

60. Drenkhan, R.; Riit, T.; Adamson, K.; Hanso, M. The earliest samples of *Hymenoscyphus albidus* vs. *H. fraxineus in Estonian mycological herbaria. Mycol. Prog.* **2016**, *15*, 835–844. [CrossRef]
61. Hanso, M.; Drenkhan, R. Diplodia pinea is a new pathogen on Austrian pine (*Pinus nigra*) in Estonia. *Plant Pathol.* **2009**, *58*, 797. [CrossRef]
62. Hanso, M.; Drenkhan, R. Lophodermium needle cast, insect defoliation and growth responses of young Scots pines in Estonia. *For. Pathol.* **2011**, *42*, 124–135. [CrossRef]
63. Lutter, R.; Drenkhan, R.; Tullus, A.; Jürimaa, K.; Tullus, T.; Tullus, H. First record of *Entoleuca mammata* in hybrid aspen plantations in hemiboreal Estonia and stand–environmental factors affecting its prevalence. *Eur. J. For. Res.* **2019**, *138*, 263–274. [CrossRef]
64. Braschler, B.; Hill, J.K. Role of larval host plants in the climate-driven range expansion of the butterfly *Polygonia c-album*. *J. Anim. Ecol.* **2007**, *76*, 415–423. [CrossRef]
65. Hemery, G.E.; Clark, J.R.; Aldinger, E.; Claessens, H.; Malvolti, M.E.; O'Connor, E.; Raftoyannis, Y.; Savill, P.S.; Brus, R. Growing scattered broadleaved tree species in Europe in a changing climate: A review of risks and opportunities. *Forestry* **2009**, *83*, 65–81. [CrossRef]
66. Cudmore, T.J.; Björklund, N.; Carroll, A.L.; Lindgren, B.S. Climate change and range expansion of an aggressive bark beetle: Evidence of higher beetle reproduction in naïve host tree populations. *J. Appl. Ecol.* **2010**, *47*, 1036–1043. [CrossRef]
67. Lekander, B.; Bejer-Petersen, B.; Kangas, E.; Bakke, A. The distribution of bark beetles in the Nordic countries. *Acta. Entomol. Fenn.* **1977**, 32. Available online: https://www.cabdirect.org/cabdirect/abstract/19770548371 (accessed on 15 January 2021).
68. Caulton, E.; Aitken, W.; Rashid, N. Aerobiological aspects of elm (*Ulmus* spp.) in South-East Scotland in relation to elm decline from Dutch Elm disease (1976–1996). *Aerobiologia* **1998**, *14*, 147–153. [CrossRef]
69. Brand, J.M.; Young, J.C.; Silverstein, R.M. Insect Pheromones: A Critical Review of Recent Advances in Their Chemistry, Biology, and Application. In *Fortschritte der Chemie Organischer Naturstoffe Progress in the Chemistry of Organic Natural Products*; Springer Science and Business Media LLC: Berlin/Heidelberg, Germany, 1979; Volume 37, pp. 1–190.
70. Cuthbert, R.A.; Peacock, J.W. Attraction of *Scolytus multistriatus* to Pheromone-baited Traps at Different Heights. *Environ. Entomol.* **1975**, *4*, 889–890. [CrossRef]
71. Neumann, F.G.; Minko, G. Studies on the introduced smaller European elm bark beetle, *Scolytus multistriatus*, a potential vector of Dutch elm disease in Victoria. *Aust. For.* **1985**, *48*, 116–126. [CrossRef]
72. Schedl, K.E. Familie: Scolytidae (Borken- und Ambrosiakäfer). In *Die Käfer Mitteleuropas*; Freude, H., Harde, K.W., Lohse, G.A., Eds.; Goecke & Evers: Krefeld, Germany, 1981; pp. 34–99.
73. Pfeffer, A. Familie: Scolytidae. In *Die Käfer Mitteleuropas, Supplementband Mit Katalogteil*; Lohse, G.A., Lucht, W.H., Eds.; Goecke & Evers: Krefeld, Germany, 1994; pp. 153–180.
74. Pfeffer, A. *Zentral- und Westpaläarktische Borken- und Kernkäfer (Coleoptera: Scolytidae, Platypodidae)*; Pro Entomologia, c/o Naturhistorisches Museum: Basel, Switzerland, 1995; 370p.
75. Petrov, A.V.; Mandelshtam, M.Y.; Beaver, R.A. A key to species of the tribe Scolytini Latreille, 1804 (Coleoptera: Curculionidae: Scolytinae) from Russia and adjacent countries. *REJ* **2019**, *28*, 286–302. [CrossRef]
76. Voolma, K.; Õunap, H.; Süda, I. *Eesti Üraskiaste (Coleoptera, Scolytidae) Määraja (A Key of Estonian Bark Beetles (Coleoptera, Scolytidae))*; Eesti Loodusfoto: Tartu, Estonia, 1997; 43p. (In Estonian)
77. Drenkhan, T.; Voolma, K.; Adamson, K.; Sibul, I.; Drenkhan, R. The large pine weevil *Hylobius abietis* (L.) as a potential vector of the pathogenic fungus *Diplodia sapinea* (Fr.) Fuckel. *Agric. For. Entomol.* **2017**, *19*, 4–9. [CrossRef]
78. Tedersoo, L.; Bahram, M.; Põlme, S.; Kõljalg, U.; Yorou, N.S.; Wijesundera, R.L.C.; Ruiz, L.V.; Vasco-Palacios, A.M.; Thu, P.Q.; Suija, A.; et al. Global diversity and geography of soil fungi. *Science* **2014**, *346*, 1256688. [CrossRef]
79. Tedersoo, L.; Anslan, S. Towards PacBio-based pan-eukaryote metabarcoding using full-length ITS sequences. *Environ. Microbiol. Rep.* **2019**, *11*, 659–668. [CrossRef]
80. Loit, K.; Adamson, K.; Bahram, M.; Puusepp, R.; Anslan, S.; Kiiker, R.; Drenkhan, R.; Tedersoo, L. Relative performance of Oxford Nanopore MinION vs. Pacific Biosciences Sequel third-generation sequencing platforms in identification of agricultural and forest pathogens. *Appl. Environ. Microbiol.* **2019**, *85*, e01368-19. [CrossRef]
81. Tedersoo, L.; Drenkhan, R.; Anslan, S.; Morales-Rodriguez, C.; Cleary, M. High-throughput identification and diagnostics of pathogens and pests: Overview and practical recommendations. *Mol. Ecol. Resour.* **2018**, *19*, 47–76. [CrossRef]
82. Agan, A.; Drenkhan, R.; Adamson, K.; Tedersoo, L.; Solheim, H.; Børja, I.; Matsiakh, I.; Timmermann, V.; Nagy, N.E.; Hietala, A.M. The Relationship between Fungal Diversity and Invasibility of a Foliar Niche—The Case of Ash Dieback. *J. Fungi* **2020**, *6*, 150. [CrossRef]
83. Tedersoo, L.; Tooming-Klunderud, A.; Anslan, S. PacBio metabarcoding of Fungi and other eukaryotes: Errors, biases and perspectives. *New Phytol.* **2018**, *217*, 1370–1385. [CrossRef]
84. Anslan, S.; Bahram, M.; Hiiesalu, I.; Tedersoo, L. PipeCraft: Flexible open-source toolkit for bioinformatics analysis of custom high-throughput amplicon sequencing data. *Mol. Ecol. Resour.* **2017**, *17*, e234–e240. [CrossRef]
85. Schloss, P.D.; Westcott, S.L.; Ryabin, T.; Hall, J.R.; Hartmann, M.; Hollister, E.B.; Lesniewski, R.A.; Oakley, B.B.; Parks, D.H.; Robinson, C.J.; et al. Introducing mothur: Open-Source, Platform-Independent, Community-Supported Software for Describing and Comparing Microbial Communities. *Appl. Environ. Microbiol.* **2009**, *75*, 7537–7541. [CrossRef] [PubMed]
86. Edgar, R.C.; Haas, B.J.; Clemente, J.C.; Quince, C.; Knight, R. UCHIME Improves Sensitivity and Speed of Chimera Detection. *Bioinformatics* **2011**, *27*, 2194–2200. [CrossRef] [PubMed]

87. Bengtsson-Palme, J.; Ryberg, M.; Hartmann, M.; Branco, S.; Wang, Z.; Godhe, A.; De Wit, P.J.G.M.; Sánchez-García, M.; Ebersberger, I.; De Sousa, F.; et al. Improved software detection and extraction of ITS1 and ITS2 from ribosomal ITS sequences of fungi and other eukaryotes for analysis of environmental sequencing data. *Methods Ecol. Evol.* **2013**, *4*, 914–919. [CrossRef]
88. Kõljalg, U.; Nilsson, R.H.; Abarenkov, K.; Tedersoo, L.; Taylor, A.F.S.; Bahram, M.; Bates, S.T.; Bruns, T.D.; Bengtsson-Palme, J.; Callaghan, T.M.; et al. Towards a unified paradigm for sequence-based identification of fungi. *Mol. Ecol.* **2013**, *22*, 5271–5277. [CrossRef] [PubMed]
89. Kühnholz, S.; Borden, J.H.; Uzunovic, A. Secondary Ambrosia Beetles in Apparently Healthy Trees: Adaptations, Potential Causes and Suggested Research. *Integr. Pest Manag. Rev.* **2001**, *6*, 209–219. [CrossRef]
90. Speranza, S.; Bucini, D.; Paparatti, B. New observation on biology of european shot-hole borer [xyleborus dispar (f.)] on hazel in northern latium (central italy). *Acta Hortic.* **2009**, *845*, 539–542. [CrossRef]
91. Deyrup, M.; Atkinson, T. Comparative Biology of Temperate and Subtropical Bark and Ambrosia Beetles (Coleoptera: Scoly-tidae, Platypodidae) in Indiana and Florida. *Gt Lakes Entomol.* **1987**, *20*, 59.
92. Steininger, M.S.; Hulcr, J.; Igut, M.; Lucky, A.; Sigut, M. Simple and Efficient Trap for Bark and Ambrosia Beetles (Coleoptera: Curculionidae) to Facilitate Invasive Species Monitoring and Citizen Involvement. *J. Econ. Entomol.* **2015**, *108*, 1115–1123. [CrossRef]
93. Rabaglia, R.J.; Dole, S.A.; Cognato, A.I. Review of American Xyleborina (Coleoptera: Curculionidae: Scolytinae) Occurring North of Mexico, with an Illustrated Key. *Ann. Entomol. Soc. Am.* **2006**, *99*, 1034–1056. [CrossRef]
94. Chang, R.; Duong, T.A.; Taerum, S.J.; Wingfield, M.J.; Zhou, X.; De Beer, Z.W. Ophiostomatoid fungi associated with conifer-infesting beetles and their phoretic mites in Yunnan, China. *MycoKeys* **2017**, *28*, 19–64. [CrossRef] [PubMed]
95. Brasier, C.M.; Kirk, S.A. Designation of the EAN and NAN races of *Ophiostoma novo-ulmi* as subspecies. *Mycol. Res.* **2001**, *105*, 547–554. [CrossRef]
96. Mandelshtam, M.Y.; Popovichev, B.G. Annotated list of bark beetle species (Coleoptera, Scoytidae) of the Leningrad Region. *Entomol. Rev.* **2000**, *80*, 887–903.
97. Mandelshtam, M.Y.; Selikhovkin, A.V. Bark and Ambrosia Beetles (Coleoptera, Curculionidae: Scolytinae) of Northwest Russia: History of the Study, Composition and Genesis of the Fauna. *Entomol. Rev.* **2020**, *100*, 1–27. [CrossRef]
98. Zolk, K. Kodumaa ürasklased (Ipidae) ühes lühikese ülevaatega nende bionoomiast ja levimisest Eestis (Native bark beetles (Ipidae) together with a brief overview of their bionomy and distribution in Estonia). *Eesti Metsanduse Aastaraam. Est. Yearb.* **1932**, *6*, 127–176. (In Estonian)
99. Voolma, K.; Süda, I. Rare species of Cerambycidae and Scolytidae (Coleoptera) in Estonia. In Proceedings of the XXIV Nordic Congress of Entomology, Tartu, Estonia, 8–11 August 1997; pp. 187–192.
100. Shcherbakova, L.N.; Mandelshtam, M.Y. Vyazy Sankt Peterburga: Posle tret'yego zvonka (Elms of Saint Petersburg: After the third ring). In Proceedings of the International Conference the Kataev Memorial Readings—VIII, St. Peterburg, Russia, 18–20 November 2014; Musolin, D.L., Selikhovkin, A.V., Eds.; Saint Petersburg State Forest Technical University: Saint Petersburg, Russia, 2014. (In Russian).
101. Kristian, J. Mõnest uuest üraskiliigist Eestis (About some new beetle species in Estonia). *Eesti Mets Estonian For.* **1937**, *17*, 399. (In Estonian)
102. Leius, K. Täiendavaid andmeid ürasklaste (Ipidae) esinemise kohta Eestis (Additional data on the occurrence of bark beetles (Ipidae) in Estonia). *Eesti Metsanduse Aastaraam. Est. Yearb.* **1939**, *9*, 318–328. (In Estonian)
103. Voolma, K.; Õunap, H.; Süda, I. *Eesti Putukate Levikuatlas, 2: Ürasklased—Scolytidae (Distribution Maps of Estonian Insects, 2: Scolytidae)*; Eesti Loodusfoto: Tartu, Estonia, 2000; 84p. (In Estonian)
104. Voolma, K.; Mandelshtam, M.; Shcherbakov, A.; Yakovlev, E.; Õunap, H.; Süda, I.; Popovichev, B.; Sharapa, T.; Galasjeva, T.; Khairetdinov, R.; et al. Distribution and spread of bark beetles (Coleoptera: Scolytidae) around the Gulf of Finland: A comparative study with notes on rare species of Estonia, Finland and North-Western Russia. *Entomol. Fenn.* **2004**, *15*, 198–210. [CrossRef]
105. Voolma, K.; Süda, I.; Õunap, H. New records of bark beetles (Coleoptera, Scolytidae) from Estonia. *Proc. Est. Acad. Sci. Biol. Ecol.* **1998**, *47*, 73–78.
106. Süda, I. Jalaka-maltsaürask (*Scolytus triarmatus* (Eggers, 1912))—uus üraskiliik Baltikumis *Scolytus triarmatus* (Eggers, 1912)—new bark beetle in the Baltics. *Metsanduslikud Uurim.* **2006**, *44*, 112–117. (In Estonian)
107. Mandelshtam, M.Y.; Khayretdinov, R.R. Additions to the list of species of bark beetles (Coleoptera, Curculionidae: Scolytinae) of the Leningrad Region (Dopolneniya k spisku vidov koroyedov (Coleoptera, Curculionidae: Scolytinae) Leningradskoy oblasti. *Entomol. Rev.* **2017**, *86*, 512–521. (In Russian)
108. Petrov, A.V.; Nikitsky, N.B. Scolytid fauna (Coleoptera, Scolytidae) of Moscow Province. *Entomol. Obozr.* **2001**, *80*, 353.
109. Vlasov, D.V.; Mandelshtam, M.Y. Elm bark beetles of the genus *Scolytus* Geoffroy, 1762 (Coleoptera: Scolytidae) as new serious pests in the parks of Yaroslavl and Saint Petersburg. In Proceedings of the Fitosanitarnoe Ozdorovlenie Ekosistem. Materialy Vtorogo Vserossiiskogo s"Ezda Po Zashchite Rastenii: V 2 Tomakh (Phytosanitary Improvement of Ecosystems: Proceedings of the 2nd All-Russian Plant Protection Congress in Two Volumes, St. Petersburg, FL, USA, 9–11 September 2005; Volume 1, p. 262. (In Russian).
110. Ganley, R.J.; Bulman, L.S. Dutch elm disease in New Zealand: Impacts from eradication and management programmes. *Plant Pathol.* **2016**, *65*, 1047–1055. [CrossRef]

111. Süda, I. Metsamardikate (Coleoptera) uued liigid Eestis (New woodland beetle species (Coleoptera) in Estonian fauna). *For. Stud.* **2009**, *50*, 98–114. (In Estonian) [CrossRef]
112. Biedermann, P.H. Observations on sex ratio and behavior of males in Xyleborinus saxesenii Ratzeburg (Scolytinae, Coleoptera). *ZooKeys* **2010**, *56*, 253–267. [CrossRef]
113. Salmane, I.; Ciematnieks, R.; Ozoliņa-Pole, L.; Ralle, B.; Ievinsh, G. Investigation of European shot-hole borer, Xyleborus dispar (Coleoptera, Scolytidae), in apple orchards of Latvia. In Proceedings of the International Scientific and Practical Conference, Rezekne, Latvia, 18–20 June 2015; Volume 2, pp. 256–260.
114. Süda, I. Metsamardikate (Coleoptera) uued liigid Eestis. 2 (New woodland beetle species (Coleoptera) in Estonian fauna. 2). *For. Stud.* **2016**, *64*, 51–69. (In Estonian) [CrossRef]
115. Süda, I. Mardikauurimisest viimastel aastakümnetel (1990–2009) (Coleoptera studies in recent decades (1990−2009)). In *Year-Book of the Estonian Naturalists' Society*, 86th ed.; Kull, T., Liira, J., Sammul, M., Eds.; Eesti Looduseuurijate Seltsi Aastaraamat; Eesti Looduseuurijate Selts: Tartu, Estonia, 2011; pp. 225–229. (In Estonian)
116. Helland, I.S.; Hoff, J.M.; Anderbrant, O. Attraction of bark beetles (Coleoptera: Scolytidae) to a pheromone trap. *J. Chem. Ecol.* **1984**, *10*, 723–752. [CrossRef]
117. Bouget, C.; Brustel, H.; Brin, A.; Valladares, L. Evaluation of window flight traps for effectiveness at monitoring dead wood-associated beetles: The effect of ethanol lure under contrasting environmental conditions. *Agric. For. Entomol.* **2009**, *11*, 143–152. [CrossRef]
118. Wegensteiner, R.; Wermelinger, B.; Herrmann, M. Natural Enemies of Bark Beetles: Predators, Parasitoids, Pathogens, and Nematodes. In *Bark Beetles*; Academic Press: Cambridge, MA, USA, 2015; pp. 247–304. [CrossRef]

IV

Jürisoo, L., Padari, A. Drenkhan, R. 2021. Jalakasurma levikust ja ohtlikkusest Eestis (Spread and riskiness of Dutch elm disease in Estonia). Forestry Studies/Metsanduslikud Uurimused, 74. [In Estonian]. DOI: 10.2478/fsmu-2021-0006.

Jalakasurma levikust ja ohtlikkusest Eestis

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Jürisoo, L., Padari, A., Drenkhan, R. Spread and riskiness of Dutch elm disease in Estonia.

Abstract. This review provides an overview of and describes the current situation of Dutch elm disease (DED), which is one of the most devastating diseases for elms worldwide and in Estonia. It is known that in Estonia DED's agent *Ophiostoma ulmi* has been damaging elms since the 1930s. Today a new species *Ophiostoma novo-ulmi* is considered to be an agent of DED. Since 2013 the current epidemic has been recorded in most of the counties of Estonia. The both known DED agents, *Ophiostoma novo-ulmi* subsp. *novo-ulmi* and *O. novo-ulmi* subsp. *americana* were molecularly detected on *Ulmus* spp. Additionally, the first time one hybrid pathogen of the subspecies (*americana* x *novo-ulmi*) was identified in northern Estonia. Also, the health status of elms and the potential vector agents of the pathogen are discussed and recommendations for disease management are provided.

Keywords: *Ulmus* spp., DED, *Ophiostoma novo-ulmi* subsp. *novo-ulmi*, *Ophiostoma novo-ulmi* subsp. *americana*, invasive species, hybrid pathogen, pathogen vector.

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Sissejuhatus

Jalaka perekonna looduslikud esindajad Eestis on harilik jalakas (*Ulmus glabra* Huds.) ja künnapuu (*U. laevis* Pall.). Mõlemat liiki leidub kogu Eestis, vähesel määral metsapuudena, kuid see-eest sageli haljastuses, eriti palju ajaloolistes parkides nii linnades (Aaspõllu, 1999; Kaar, 2011) kui ka maapiirkonnas (Abner *et al.*, 2007; Abner *et al.*, 2012). Laialeheliste lehtpuude hulka kuuluvad harilikud jalakad ja künnapuud on ökoloogiliselt olulised liigid (Martín *et al*., 2019; Thor *et al*., 2010) – nendega on kohastunud elama mitu sambliku- ja seeneliiki. Näiteks on harilikult jalakalt leitud 39 erinevat samblikku, kusjuures kaks neist on ohualtid ja üks ohustatud liik (Jüriado *et al.*, 2009). Ka kaks ohustatud seeneliiki nagu roosa võrkheinik (*Rhodotus palmatus*) ja jalaka-tubakanahkis (*Hymenochaete ulmicola*) sõltuvad eelkõige jalakatest (Corfixen & Parmasto, 2005; Kalamees, 2011). Looduskaitseliste tegevuskavade koostamisel jalakatest sõltuvatele liikidele, nt roosa võrkheiniku kaitse tegevuskava (Sell, 2019), tuleb arvestada muuseas ka jalakasurma levikuga.

Nagu mujal nii ohustab ka meie jalakaid jalakasurm (*Ophiostoma ulmi sensu lato*), olles üheks kõige laastavamaks jalakaliikide haiguseks kogu maailmas (Brasier, 1991; Brasier & Webber, 2019). Kahe haiguspuhangu jooksul 20. sajandil on jalakasurm hävitanud maailmas miljardeid

jalakaid (Phillips & Burdekin, 1992; Herald, 2019). Rootsis on jalakasurma tõttu kohalike liikide (*U. glabra*, *U. laevis*, *U. minor*) arvukus vähenenud sellisel määral, et need on võetud punasesse nimistusse (Hallin, 2010). Peterburis anti ainuüksi 2015. aastal välja üle 5000 raieloa surevate jalakate eemaldamiseks (Jürisoo *et al.*, 2021).

Jalakasurma tekitaja jalaka-siugsuu (*Ophiostoma ulmi*) on kottseen siugsuudmeliste (*Ophiostomataceae*) sugukonnast (eElurikkus, 2019). Praeguseks kõikjal levinud jalakasurma tekitaja uut liiki *Ophiostoma novo-ulmi* nimetame eesti keeles „uus jalaka-siugsuu". Jalakasurm on agressiivne, ta võib täiskasvanud puu surmata isegi 1-2 aastaga (Phillips & Burdekin, 1992; Schmidt, 2006).

Eestis on leitud jalakasurma (tekitajaks tollal arvatud *O. ulmi)* juba eelmise sajandi 30-ndatel aastatel (Lepik, 1940a; Kaar, 2011). Järgmist haiguspuhangut täheldati Eestis esmakordselt eelmise sajandi viimastel kümnenditel (M. Hanso, suulised andmed), ehkki tollal peeti meil seda haigust väheoluliseks (Kalamees & Sõmermaa, 2000). Jalakasurma levik hoogustus aga taas alates uue sajandi teisest kümnendist. Jalakasurma uut levikut võisid soodustada nii kliima soojenemine kui ka globaalne kaubandus (Brasier, 2008; Rytkönen *et al.*, 2008; Rytkönen *et al.*, 2011; Drenkhan *et al.*, 2017a; Ghelardini *et al.*, 2017), eelkõige aga patogeeni uue liigi levimine.

Jalakasurma tekitavaid seeni levitavad eelkõige vektorputukad, samuti levib patogeen lokaalselt juurekontakti kaudu (Gibbs, 1978) ning kaudselt soodustab haiguse levikut jalakate lai leviala maailmas. Jalakaid on märkimisväärselt kasutatud alleede ja parkide rajamisel, kus neid kasvab rohkesti koos. Jalakate üldise seisundi kohta Eestis enne 2014. aastat oli info lünklik. Nüüdseks on siinse töö raames uuritud jalakaid ja nende tervislikku seisundit mõjutavat jalakasurma kõikides Eesti maakondades. Seda ohtlikku, nüüdseks uue tekitajaliigi tõttu invasiivseks peetavat metsahaigust tutvustades käsitletakse artiklis jalakasurma diagnostikat, haiguse levikut, haigusetekitajate taksonoomiat, peremeestaimede seisundi patoloogilisi muutusi ja haiguse tõrje võimalusi.

Jalakasurma diagnostika

Jalakasurma esmased tunnuseid võib märgata võras vaid üksikutel okstel. Haigus tekitab puudel trahheomükoosi tüüpi kahjustust – seene elutegevuse tõttu blokeeritakse vee ja toitainete liikumine, mille tõttu varasuvel väga lühikese aja jooksul närbuvad, kolletuvad ja kuivavad kasvufaasis olevad lehed. Kärbunud lehed jäävad esialgu võrasse ja hiljem varisevad (joonis 1).

Joonis 1. Jalakasurma esmased tunnused harilikul jalakal, haiguse tagajärjel kärbunud lehed jäävad esialgu võrasse.
Figure 1. First symptom of Dutch elm disease – wilting leaves in tree crown

Haigustunnustega oksa ristlõikel on nähtavad tumepruunid juhtkimbud täppidena või ringina koore lähedases puidus (joonis 2).

Joonis 2. Nakatunud puult lõigatud oksa ristlõikel on nähtavad tumepruunid juhtkimbud täppidena või ringina koore lähedases puidus
Figure 2. Cross section of twigs from an infected tree indicates a circle of vascular bundle as several brown dots or as a ring in xylem next to the floem

Nakatunud puus paljuneb jalakasurma tekitaja mittesuguline arengujärk pärmilaadsetele seentele sarnase pungumise teel ning valmis eosed levivad ksüleemis kiiresti koos vee ja toitainete liikumisega (Webber & Brasier, 1984). Puude ulatuslikku, massilist suremist jalakasurma tõttu võis näha Vana-Vastseliinas Lõuna-Eestis 2018. aasta suvel (vt joonis 3).

Joonis 3. Jalakasurma tõttu surnud harilikud jalakad Vana-Vastseliinas, Võrumaal 2018. aastal
Figure 3. In 2018, *Ulmus glabra* trees were killed by DED in Vana-Vastseliina, South Estonia

Jalakasurm maailmas ja lähiriikides

Jalakasurma esimene tekitaja *Ophiostoma ulmi* (Buisman) Melin & Nannf. (varasemad sünonüümid *Graphium ulmi* M.B. Schwartz, *Ceratostomella ulmi* Buisman, *Ceratocystis ulmi* (Buisman) C. Moreau, *Pesotum ulmi* (M.B. Schwartz) J.L. Crane & Schokn) (Lepik, 1940a; Brasier & Buck, 2001), mille täpne päritolu on teadmata, registreeriti 1918. a Lääne-Euroopas (Brasier, 1979). Mõningatel andmetel oli jalakate suremist märgatud Lääne-Euroopas veel varemgi (Santini & Faccoli, 2013). Hollandis kirjeldati jalakasurma esmalt 1921. aastal, kust on pärit ka haiguse ingliskeelne nimi – *Dutch elm disease* (Clinton & McCormick, 1936). *Ophiostoma ulmi* levis 1920–1930ndatel Euroopast imporditud ja nakatunud jalakapuiduga nii Põhja-Ameerikasse kui ka Kesk-Aasiasse (Brasier, 1990).

Jalakasurma uus epideemia, mis peagi kujunes pandeemiaks, sai alguse 1970. aastatel. See hävitas jalakaid nii Euroopas, Loode- ja Kesk-Aasias kui ka Põhja-Ameerikas (Gibbs, 1978). Uue puhangu põhjustanud patogeenil *Ophiostoma novo-ulmi* tuvastati peagi seejärel kaks rassi,

mis nimetati vastavalt nende levialale Euraasia rassiks (EAN) ja Põhja-Ameerika rassiks (NAN). Hiljem nimetati needsamad seenerühmad ümber *O. novo-ulmi* alamliikideks, vastavalt *O. novo-ulmi* subsp. *novo-ulmi* ja *O. novo-ulmi* subsp. *americana* (Brasier & Kirk, 2001). Lisaks erinevale üldisele levialale erinevad need kaks alamliiki teineteisest ka molekulaarselt (Pipe *et al.*, 1997; Brasier & Kirk, 2001), kuid niisamuti ka haigusetekitaja agressiivsuselt (Brasier & Buck, 2001).

Nagu märgitud, on *Ophiostoma ulmi* ja *O. novo-ulmi* eri liigid (Pipe *et al.*, 2000) erineva ja ebaselge päritoluga (Brasier & Kirk, 2010). Need liigid erinevad teineteisest puhaskultuuride optimaalse kasvutemperatuuri, kuid ka seenemütseeli struktuuri (*colony pattern*) poolest ja, mis eriti oluline, nende geenides olevate nukleotiidide järjestuse poolest (Brasier, 1991). Esimese puhangu aegset *O. Ulmi*'t on peetud Euroopas levivatel jalakatel suhteliselt nõrgaks patogeeniks, võrreldes kaasaegse agressiivsema patogeeni *O. novo-ulmi*'ga (Brasier, 2000).

Meie naabermaadest on Soomes jalakasurma registreeritud eelmise sajandi teisel poolel (Hintikka, 1974), kuid värskemate andmete põhjal seda haigust Soomes teadaolevalt ei esine (Hannunen & Marinova-Todorova, 2016; Hantula, 2021). Rootsis Malmös kasvas 1985. aastal alleedel, aedades ja parkides kokku *ca* 33 000 jalakat, mis moodustas kõikidest tänavapuudest 25%. Peale jalakasurma esmaavastamist seal 1985. aastal kasvas jalakasurma juhtumite hulk kiiresti, eriti just alates 1995. aastast ning nii Malmö linnas kui ka lähiümbruses (Arne Mattsson, endine linnaaednik, suulised andmed). Lähiriikidest on jalakasurma tuvastatud ka Lätis (Matisone *et al.*, 2020) ja Leedus (Motiejūnaitė *et al.*, 2016), meist kaugemal Ida-Euroopas veel Tšehhis (Dvořák *et al.*, 2007), Poolas (Łakomy *et al.*, 2016), Sloveenias (Ogris, 2018) ja Horvaatias (Stančin, 2018). Vahetus läheduses Peterburis on see haigus põhjustanud massilist jalakate surma alates aastast 1995 (Jürisoo *et al.*, 2021).

Arvatavasti jõudis *O. novo-ulmi* subsp. *novo-ulmi* Ukraina Musta mere ranniku piirkonda 1940ndatel ja levis sealt edasi Lääne-Euroopasse (Brasier, 2001). Seevastu *O. novo-ulmi* subsp. *americana* levis 1940ndatel Ameerikas Suurjärvistu piirkonnast üle kogu USA ja Kanada. Euroopasse (Ühendkuningriiki) toodi viimane sisse nakatunud jalaka puiduga 1960ndatel ning levis läänest itta (Brasier, 2000; Brasier & Kirk, 2000; Gibbs & Brasier, 1973).

Seoses jalakasurma tekitaja päritolu otsingutega kirjeldati eelmise sajandi lõpus Himaalaja mäestiku piirkonnast leitud uut liiki *O. himal-ulmi* Brasier & Mehrotra, mis osutus selgelt lähedaseks jalakasurma tekitavatele liikidele. Kohalikele jalakatele polnud see liik patogeenne, vaid käitus pigem tagasihoidliku surnud kudede lagundajana (Brasier & Mehrotra, 1995). Sarnaselt käitub ka saaresurma tekitaja *Hymenoscyphus fraxineus* arvataval päritolumaal Ida-

Aasias, kus tekitab kohalikel saare liikidel vaid vähesel määral lehtede ja võrsete kahjustusi (Drenkhan *et al.*, 2017b).

Alates 1980ndatest eristati Euroopas juba ka kahe jalakasurma tekitaval alamliigil omavahelisi hübriide – *O. novo-ulmi* subsp. *novo-ulmi* x subsp. *americana* ja *O. novo-ulmi* subsp. *americana* x subsp. *novo-ulmi* (Brasier & Kirk, 2010), mis on puhastest alamliikidest kiirema kasvuga ja sellest johtuvalt ka agressiivsemad, mida täheldati enne hübriidide avastamist (Gibbs & Brasier, 1973). Balti riikidesse ja Venemaale jõudsid need hübriidid piiride avamise järel. Praeguseks on neid hübriide juba tuvastatud Norras, Rootsis (Brasier & Kirk, 2010), Leedus (Motiejūnaitė *et al.*, 2016), Lätis (Matisone *et al.*, 2020) ja Loode-Venemaal (Peterburg) (Jürisoo *et al.*, 2021). Hübriidide suurema agressiivsuse tõttu kasvab oht jalakate populatsioonile tulevikus veelgi (Ellstrand & Schierenbeck, 2000).

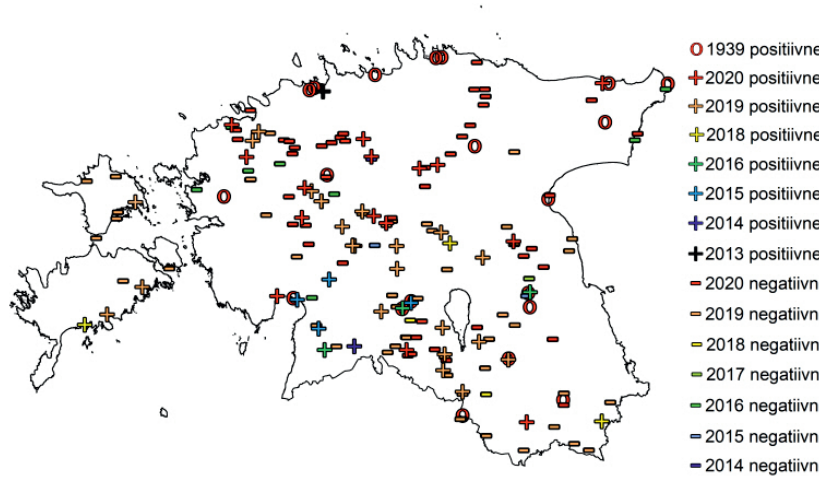
Jalakasurma tekitajate leiud Eestis

Eelmise sajandi algul kogutud informatsioon jalakasurma leviku kohta kinnitab, et see haigus oli levinud peaaegu tervel Eesti mandriosal (Lepik, 1940b). Hilisem informatsioon jalakasurma kohta pole nii süsteemne, vaid piirdub üksikute teadaannetega ajakirjanduses (Pintson, 2015; Aaspõllu, 2017; Mihkelson, 2017; Aotäht, 2018) ning märgetega parkide ja haljasalade inventeerimisdokumentides (Laas & Treumuth, 2006; Jürisoo, 2015a, 2015b; Rist, 2015). Dendroloogid on jalakatest ja künnapuudest kirjutades maininud tihti ka jalakasurma (Abner *et al.*, 2007, 2012; Kaar, 2011).

Siinse kokkuvõtva artikli koostamiseks aastatel 2013–2020 tehtud uuringud näitavad, et jalakasurm on Eestis endiselt laialt levinud (joonis 4). Piirkonnad, kus eelmise sajandi esimesel poolel jalakasurma täheldati (Lepik, 1940b), olid enamasti nakatunud ka 2020. aastal, kuid leiti ka mõningaid erinevusi (joonis 4). Eelmisel sajandil ei tuvastatud jalakasurma saartelt ja Eesti kagupoolseimast nurgast Vastseliinast. Kuid Narvas, Mustvees, Võrus ja Raplas, kus jalakasurma leiti esimese epideemia ajal, pole sellel sajandil jalakasurma tuvastatud. Heimtalis Raudna ürgoru vasemal kaldal on ligi kahel hektaril kasvanud jalakapuistust alles vaid mõned üksikud harilikud jalakad ning künnapuud. Oru pargis Ida-Virumaal Kirde-Eestis esines jalakasurma eelmise sajandi alguses, kuid 2016. aastal sealt jalakasurma ei tuvastatud, 2020. aastal leiti sealt aga taas nakatunud jalakaid. Kui jalakasurm jäi nimetatud puistus spetsialistile märkamatuks, siis sellel võib olla erinevaid võimalikke põhjusi. Näiteks võis tegemist olla perioodiga, mil toodi kusagile lähikonda jalakasurma tekitaja nakkusega istutusmaterjali või tõid nakkuse vektorputukad, kust haigus levis ka kohalikele vanematele puudele.

Nii nagu mujal maailmas tunti ka Eestis esmalt jalakasurma tekitajat nimetuse all *Ophiostoma ulmi* Buisman (Lepik, 1940a). Huvitav on seejuures märkida, et EPPO (Euroopa ja Vahemeremaade taimekaitse organisatsiooni) nimekirjas on see nimetus esmakordselt registreeritud 1979. aastal aga just Eestis, samal ajal ka teistes Balti riikides (EPPO Global Database, 2019).

Sajandi lõpuks oli *O. ulmi* Eestis asendunud palju agressiivsema liigiga *O. novo-ulmi* Brasier (Hanso & Drenkhan, 2007; Drenkhan *et al.*, 2017) nii nagu see oli juhtunud ka suuremas osas Euroopas ja Põhja-Ameerikas (Brasier, 2000a).



Joonis 4. Jalakasurma leiud Eestist vastavalt aastatel 1939 ja 2013-2020, „O" – 1939 aasta positiivsed leiud, „+" – positiivsed leiud aastatel 2013-2020, „-„ – negatiivsed leiud aastatel 2013-2020 (aluskaart Haldus- ja asustusjaotus, 2020).
Figure 4. Records of DED in 1939 and 2013-2020 in Estonia, „O" – 1939 positive records, „+" – positive records in 2013-2020, „-„ – negative records in 2013-2020 (base map Haldus- ja asustusjaotus, 2020).

Aastatel 2013–2016 Eestist kogutud ja analüüsitud proovide põhjal selgus, et siin on levinud *Ophiostoma novo-ulmi* mõlemad alamliigid (Jürisoo et al., 2019). Ühestki nii varem teadaolevast leiukohast kui ka uutest leiukohtadest *O. Ulmi*'t tuvastatud pole. Kahjuks puuduvad igasugused andmed vahepealsest perioodist, mistõttu ei oska ka ennustada, millal toimus üleminek ühelt tekitajalt teisele. Euroopa päritolu alamliiki *O. novo-ulmi* subsp. *novo-ulmi* leiti sel perioodil Tartu-, Viljandi- ja Pärnumaalt, patogeensemat Ameerika päritolu alamliiki (*O. novo-ulmi* subsp. *americana*) tuvastati sel ajal aga vaid Tallinnast (Jürisoo *et al*., 2019).

Kolmel aastal hinnati kokku 990 puud – 2018. aastal kaheksas, 2019. aastal 80. ja 2020. aastal 72. juhuslikult valitud jalakate kasvukohas üle Eesti. Kokku korjati sel perioodil 280 proovi, mis vastasid jalakasurma tunnustele – lehtede kolletumine või närbumine. Neist isoleeriti 108 jalakasurma tekitaja puhaskultuuri, mille rDNAst sekveneeriti ITS järjestused täpseks liigi tuvastamiseks. Kõikide puhaskultuuride andmed deponeeriti Tartu seenekultuuride kollektsiooni (Tartu Fungal Culture Collection, TFC) EMÜ metsapatoloogia labori seente puhaskultuuride alamkogusse (PAT), neist 52 määratud rDNA ITS järjestused deponeeriti rahvusvahelises Geenipangas (tabel 1). Jalakasurm tuvastati varasemas teadustöös kirjeldatud metoodika alusel (Jürisoo *et al.*, 2019; Konrad *et al.*, 2002). Viimaste proovide analüüside põhjal tuvastati *O. novo-ulmi* subsp. *novo-ulmi* lisaks varasemale (2013–2016) Harjumaal, Saaremaal, Võrumaa kaguosas, Jõgevamaal, Valgamaal ning Järvamaal – kokku 91 proovis (Joonis 4). 2019. aastal leiti patogeeni alamliiki subsp. *americana* juba ka Hiiumaalt ning 2020. aastal Koselt Harjumaalt ja Jalaselt Raplamaalt kogutud hariliku jalaka proovides, kokku 16 proovis (tabel 1).

Uue arenguna jalakasurma sündroomis tuvastati 2020. aastal esimest korda jalakasurma tekitaja alamliikide vahelist hübriidi (*Ophiostoma novo-ulmi* subsp. *americana x novo-ulmi*) ühest Kosel (Harjumaal) korjatud hariliku jalaka proovist, seene isolaat seenekogus tähistati numbriga TFC101157 (vt tabel 1).

Tabel 1. Perioodil 2018–2020 jalakatelt kogutud haigusproovid ja nende uurimise tulemused molekulaarsete määrangute järgi
Table 1. *Symptomatic samples collected from elms in the period 2018–2020 and molecularly detected DED agents*

Maakond	Proovi kogumise				Peremeestaim	Proovi nr[1]	Seene kood TFC kollek-tsioonis[2]	Geenipanga kood, ITS järgi	*Ophiostoma novo-ulmi* alamliik[3]	
	kuupäev	piirkond							*col1*[4]	*cu+Hph I*[5]
Harju	29.06.19	Padise	maa	park	*Ulmus glabra*	17743	TFC101145	MW575298	*nu*[6]	*nu*
	30.06.19	Audevälja	maa	park	*U. glabra*	17745	TFC101146	MW575299	*nu*	*nu*
	03.07.19	Tallinn	linn	park	*U. glabra*	17750	TFC101147		*am*[7]	*am*
	03.07.19	Tallinn	linn	park	*U. glabra*	18530	TFC101148		*am*	*am*
	03.07.19	Tallinn	linn	park	*U. glabra*	19653	TFC101149		*am*	*am*
	17.07.19	Tallinn	linn	park	*U. glabra*	18526	TFC101150		*am*	*am*
	17.07.19	Tallinn	linn	tänav	*U. glabra*	18525	TFC101151		*am*	*am*
	07.08.19	Tallinn	linn	tänav	*U. glabra*	18923	TFC101152		*am*	*am*
	04.09.19	Tallinn	linn	tänav	*U. glabra*	19213	TFC101153		*am*	*am*
	21.06.20	Tallinn	linn	park	*U. glabra*	25714	TFC101154		*am*	*am*
	21.06.20	Tallinn	linn	park	*U. glabra*	25715	TFC101155		*am*	*am*
	21.06.20	Tallinn	linn	tänav	*U. glabra*	25716	TFC101156		*am*	*am*
	04.07.20	Kose	maa	tänav	*U. glabra*	26042	TFC101157	MW575325	*am*	*nu*
	04.07.20	Kose	maa	tänav	*U. glabra*	26043	TFC101158		*am*	*am*
	04.07.20	Kose	maa	tänav	*U. glabra*	26044	TFC101159		*am*	*am*
	04.07.20	Kose	maa	tänav	*U. glabra*	26045	TFC101160		*am*	*am*
	04.07.20	Kose	maa	tänav	*U. glabra*	26046	TFC101161		*am*	*am*
	31.07.20	Triigi	maa	park	*U. glabra*	26205	TFC101162	MW575333	*nu*	*nu*
	31.07.20	Triigi	maa	park	*U. glabra*	26206	TFC101163		*nu*	*nu*
	31.07.20	Triigi	maa	park	*U. glabra*	26207	TFC101164		*nu*	*nu*
	31.07.20	Triigi	maa	park	*U. glabra*	26209	TFC101165		*nu*	*nu*
	31.07.20	Triigi	maa	park	*U. glabra*	26210	TFC101166		*nu*	*nu*
	31.07.20	Padise	maa	park	*U. glabra*	26208	TFC101167	MW575334	*nu*	*nu*
	01.08.20	Vihterpalu	maa	park	*U. glabra*	26165	TFC101168	MW575332	*nu*	*nu*
Hiiu	07.09.19	Suuremõisa	maa	park	*U. glabra*	19217	TFC101169	MW575315	*am*	*am*
Ida-Viru	24.06.20	Toila	maa	park	*U. glabra*	25718	TFC101170	MW575324	*nu*	*nu*
	24.06.20	Toila	maa	park	*U. glabra*	25719	TFC101171		*nu*	*nu*
Jõgeva	29.07.18	Põltsamaa	linn	tänav	*U. glabra*	14903	TFC101172	MW575294	*nu*	*nu*
	29.07.18	Põltsamaa	linn	park	*U. glabra*	14913	TFC101173		*nu*	*nu*

Maakond	Proovi kogumise				Peremeestaim	Proovi nr[1]	Seene kood TFC kollek-tsioonis[2]	Geenipanga kood, ITS järgi	*Ophiostoma novo-ulmi* alamliik[3]	
	kuupäev	piirkond							*col1*[4]	*cu+Hph I*[5]
	29.07.18	Põltsamaa	linn	park	*U. glabra*	14914	TFC101174		*nu*	*nu*
	20.06.19	Põltsamaa	linn	park	*U. glabra*	17728	TFC101175		*nu*	*nu*
	03.07.19	Põltsamaa	linn	tänav	*U. glabra*	19961	TFC101176	MW575322	*nu*	*nu*
	03.07.19	Adavere	maa	park	*U. glabra*	17753	TFC101177	MW575302	*nu*	*nu*
	21.06.19	Puurmani	maa	park	*U. glabra*	17730	TFC101178	MW575296	*nu*	*nu*
	21.06.19	Puurmani	maa	park	*U. laevis*	17735	TFC101179		*nu*	*nu*
	28.07.20	Luua	maa	mets	*U. glabra*	26145	TFC101180	MW575331	*nu*	*nu*
	06.09.20	Luua	maa	park	*'Camperdownii'*	26488	TFC101181	MW575341	*nu*	*nu*
	06.09.20	Luua	maa	park	*U. glabra*	26489	TFC101182	MW575342	*nu*	*nu*
Järva	12.08.19	Villevere	maa	park	*U. glabra*	19215	TFC101183	MW575314	*nu*	*nu*
	12.08.19	Villevere	maa	park	*U. glabra*	18928	TFC101184		*nu*	*nu*
	05.09.19	Käru	maa	tänav	*U. glabra*	19947	TFC101185	MW575317	*nu*	*nu*
	05.09.19	Käru	maa	tänav	*U. laevis*	19948	TFC101186	MW575318	*nu*	*nu*
	21.07.20	Järva-Jaani	maa	park	*U. glabra*	26133	TFC101187	MW575328	*nu*	*nu*
	21.07.20	Roosna-Alliku	maa	tee	*U. glabra*	26134	TFC101188	MW575329	*nu*	*nu*
	21.07.20	Roosna-Alliku	maa	tee	*U. glabra*	26135	TFC101189		*nu*	*nu*
	21.07.20	Roosna-Alliku	maa	tee	*U. glabra*	26136	TFC101190		*nu*	*nu*
	21.07.20	Roosna-Alliku	maa	tee	*U. glabra*	26139	TFC101191		*nu*	*nu*
	21.07.20	Roosna-Alliku	maa	tee	*U. glabra*	26140	TFC101192		*nu*	*nu*
	21.07.20	Roosna-Alliku	maa	tee	*U. glabra*	26142	TFC101193	MW575330	*nu*	*nu*
	21.07.20	Roosna-Alliku	maa	tee	*U. glabra*	26141	TFC101194		*nu*	*nu*
	08.08.20	Kolu	maa	tee	*U. glabra*	26217	TFC101195	MW575338	*nu*	*nu*
	08.08.20	Kolu	maa	tee	*U. glabra*	26218	TFC101196		*nu*	*nu*
	08.08.20	Kolu	maa	tee	*U. glabra*	26219	TFC101197		*nu*	*nu*

Maakond	Proovi kogumise				Peremeestaim	Proovi nr[1]	Seene kood TFC kollek-tsioonis[2]	Geenipanga kood, ITS järgi	*Ophiostoma novo-ulmi* alamliik[3]	
	kuupäev	piirkond							*col1*[4]	*cu+Hph I*[5]
	08.08.20	Laupa	maa	park	*U. glabra*	26220	TFC101198	MW575339	*nu*	*nu*
	08.08.20	Laupa	maa	park	*U. glabra*	26221	TFC101199		*nu*	*nu*
Lääne	01.08.20	Kuijõe	maa	park	*U. glabra*	26211	TFC101200	MW575335	*nu*	*nu*
	01.08.20	Kuijõe	maa	park	*U. glabra*	26212	TFC101201		*nu*	*nu*
	01.08.20	Kuijõe	maa	park	*U. glabra*	26213	TFC101202		*nu*	*nu*
	02.08.20	Kuijõe	maa	park	*U. laevis*	26214	TFC101203	MW575336	*nu*	*nu*
Pärnu	11.06.19	Tihemetsa	maa	park	*U. laevis*	19208	TFC101204	MW575310	*nu*	*nu*
	28.06.19	Tihemetsa	maa	park	*U. glabra*	19964	TFC101205	MW575323	*nu*	*nu*
	29.06.19	Pärnu	linn	tänav	*U. glabra*	17737	TFC101206	MW575297	*nu*	*nu*
	13.08.19	Tori	maa	park	*U. glabra*	18921	TFC101207	MW575308	*nu*	*nu*
	13.08.19	Tori	maa	park	*U. glabra*	18924	TFC101208		*nu*	*nu*
	13.08.19	Pärnu	linn	park	*U. glabra*	18926	TFC101209		*nu*	*nu*
	13.08.19	Tori	maa	park	*U. glabra*	19214	TFC101210		*nu*	*nu*
	01.09.19	Vändra	maa	park	*U. glabra*	19211	TFC101211	MW575312	*nu*	*nu*
	19.08.20	Audru	maa	park	*U. glabra*	26487	TFC101212	MW575340	*nu*	*nu*
Rapla	30.06.19	Valli	maa	tee	*U. glabra*	17746	TFC101213	MW575300	*nu*	*nu*
	30.06.19	Kabala	maa	park	*U. glabra*	17748	TFC101214	MW575301	*nu*	*nu*
	05.09.19	Valli	maa	park	*U. glabra*	19954	TFC101215	MW575321	*nu*	*nu*
	02.08.20	Kirna	maa	park	*U. glabra*	26222	TFC101216		*nu*	*nu*
	08.08.20	Jalase	maa	park	*U. glabra*	26216	TFC101217	MW575337	*am*	*am*
Saare	04.08.18	Kuressaare	linn	tänav	*U. glabra*	14917	TFC101218	MW575295	*nu*	*nu*
	07.09.19	Uduvere	maa	tee	*U. glabra*	19949	TFC101219	MW575319	*nu*	*nu*
	06.09.19	Pihtla	maa	park	*U. glabra*	19953	TFC101220	MW575320	*nu*	*nu*
Tartu	26.07.18	Tartu	linn	tänav	*U. glabra*	14918	TFC101221		*nu*	*nu*
	25.07.19	Rõngu	maa	park	*U. glabra*	18537	TFC101222	MW575306	*nu*	*nu*
	28.07.19	Tartu	linn	park	*U. glabra*	19216	TFC101223		*nu*	*nu*
	15.08.20	Tartu	linn	park	*U. glabra*	26485	TFC101224		*nu*	*nu*
	15.08.20	Tartu	linn	park	*U. glabra*	26486	TFC101225		*nu*	*nu*
Valga	19.07.19	Helme	maa	park	*U. glabra*	18521	TFC101226	MW575303	*nu*	*nu*
	19.07.19	Helme	maa	park	*U. glabra*	18532	TFC101227	MW575305	*nu*	*nu*
	19.07.19	Helme	maa	park	*U. glabra*	18540	TFC101228	MW575307	*nu*	*nu*

Maakond	Proovi kogumise				Peremeestaim	Proovi nr[1]	Seene kood TFC kollek-tsioonis[2]	Geenipanga kood, ITS järgi	*Ophiostoma novo-ulmi* alamliik[3]	
	kuupäev	piirkond							*col1*[4]	*cu+Hph I*[5]
	19.07.19	Helme	maa	park	*U. glabra*	18541	TFC101229		*nu*	*nu*
	19.07.19	Helme	maa	park	*U. glabra*	18542	TFC101230		*nu*	*nu*
	25.07.19	Riidaja	maa	park	*U. glabra*	18523	TFC101231	MW575304	*nu*	*nu*
	25.07.19	Hummuli	maa	park	*U. glabra*	18524	TFC101232		*nu*	*nu*
	02.09.19	Kibena	maa	puistu	*U. glabra*	19220	TFC101233	MW575316	*nu*	*nu*
	02.09.19	Otepää	linn	tänav	*U. glabra*	19212	TFC101234	MW575313	*nu*	*nu*
Viljandi	20.06.19	Heimtali	maa	park	*U. glabra*	17729	TFC101235		*nu*	*nu*
	19.07.19	Viljandi	linn	tänav	*U. glabra*	18533	TFC101236		*nu*	*nu*
	13.08.19	Suure-Kõpu	maa	park	*U. glabra*	19201	TFC101237	MW575309	*nu*	*nu*
	13.08.19	Suure-Kõpu	maa	park	*U. glabra*	19203	TFC101238		*nu*	*nu*
	13.08.19	Suure-Kõpu	maa	park	*U. glabra*	19205	TFC101239		*nu*	*nu*
	13.08.19	Suure-Kõpu	maa	park	*U. glabra*	19206	TFC101240		*nu*	*nu*
	13.08.19	Suure-Kõpu	maa	park	*U. glabra*	19207	TFC101241		*nu*	*nu*
	28.08.19	Viljandi	linn	park	*U. glabra*	19209	TFC101242		*nu*	*nu*
	01.09.19	Suure-Jaani	linn	park	*U. glabra*	19210	TFC101243	MW575311	*nu*	*nu*
	16.07.20	Polli	maa	park	*U. glabra*	26129	TFC101244	MW575327	*nu*	*nu*
Võru	01.08.18	Vastseliina	maa	puistu	*U. glabra*	14889	TFC101245	MW575293	*nu*	*nu*
	01.08.18	Vastseliina	maa	puistu	*U. glabra*	14894	TFC101246		*nu*	*nu*
	01.08.18	Vastseliina	maa	puistu	*U. glabra*	14898	TFC101247		*nu*	*nu*
	13.07.19	Vastseliina	maa	park	*U. glabra*	18528	TFC101248		*nu*	*nu*
	13.07.19	Vastseliina	maa	park	*U. glabra*	18529	TFC101249		*nu*	*nu*
	05.07.20	Tsooru	maa	park	*U. glabra*	26051	TFC101250	MW575326	*nu*	*nu*
	05.07.20	Tsooru	maa	park	*U. glabra*	26052	TFC101251		*nu*	*nu*
	05.07.20	Tsooru	maa	park	*U. glabra*	26053	TFC101252		*nu*	*nu*

[1] Proovi nr – Proovi ID number, registreeritud Eesti Maaülikooli Metsapatoloogia ja -geneetika laboris / Sample ID in the collection of the Laboratory of Forest Pathology and Genetics of the Estonian University of Life Sciences.

[2] Eesti Maaülikooli seente puhaskultuuride kollektsioon /Fungal Collection in Estonian University of Life Sciences, Estonia (TFC).
[3] Kui mõlemad geenid tuvastasid erineva patogeeni alamliigi, siis on tegemist seene hübriidiga/ If both genes identified a different subspecies of the pathogen then it is considered a fungal hybrid.
[4] *col1* – *col1* tüüpi geen/the colony type gene (Konrad *et al.* 2002).
[5] *cu*+*Hph* I – *cu geeni restriktsioonanalüüs ensiümiga Hph I* / ceratoulmin gene cu digested with enzyme *Hph* I (Konrad *et al.* 2002).
[6] *nu* – *Ophiostoma novo-ulmi* subsp. *novo-ulmi.*
[7] *am* – *O. novo-ulmi* subsp. *americana.*

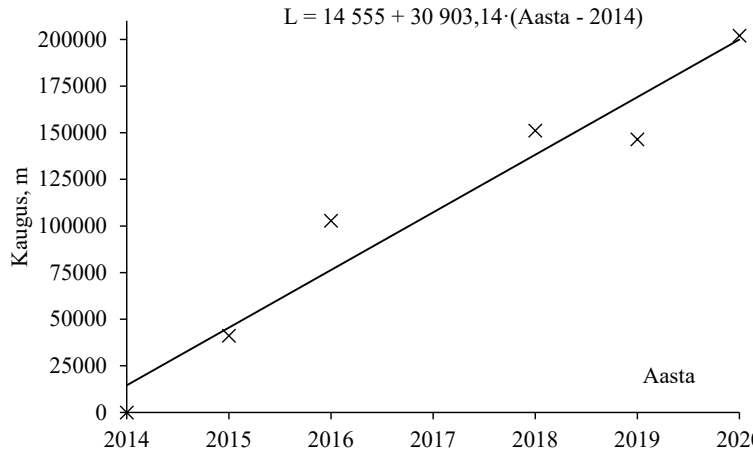
Jalakasurma tekitajate võimalik levikustsenaarium Eestis

Seirates kogu Eestit, koguti süsteemselt haigusproove perekond jalaka erinevatelt liikidelt üksikvaatlustega 2013. aastal ja perioodidel 2014–2016 (Jürisoo et al., 2019) ning 2018–2020. Tabelis 2 on esitatud jalakasurma tekitajate osas positiivsete proovide leiukohad ja nende kaugused vastava alamliigi esimesest molekulaarselt määratud leiust Eestis. Tabelis 2 ja joonisel 5 on esitatud nii empiirilised kui ka teoreetilised tulemused aastate kaupa, mille eesmärgiks oli katse jälitada taksonite levimise käiku Eestis.

Tabel 2. Jalakasurma enamlevinud tekitaja (*O. novo-ulmi* subsp. *novo-ulmi*) positiivsete leidude maksimaalne ja teoreetiline kaugus esimesest positiivsest leiukohast Tihemetsas (2014. a) ning leviku kiirus, hinnangud aastate järgi.
Table 2. *Maximum and theoretical distance of positive records of DED pathogen (O. novo-ulmi subsp. novo-ulmi) from the first positive sampling site in Tihemetsa (2014) and rate of spread by estimated years.*

Patogeeni tuvastamise aasta	Maksimaalne kaugus eelmisest positiivsest leiust, m	Teoreetiline kaugus (valem 1), m
2014	0	14555
2015	41078	45458
2016	102823	76362
2017	-	107265
2018	151173	138168
2019	146445	169071
2020	202068	199974

Jalakasurma tekitaja positiivsete proovide levimiskäigu üldistamiseks kogu Eestile teostati regressioonanalüüs. Regressioonanalüüsil saadaud determinatsioonikordaja (R^2) on 0,971, prognoosiviga 20 104 m ja olulisuse tõenäosus (p-väärtus) 0,0012.

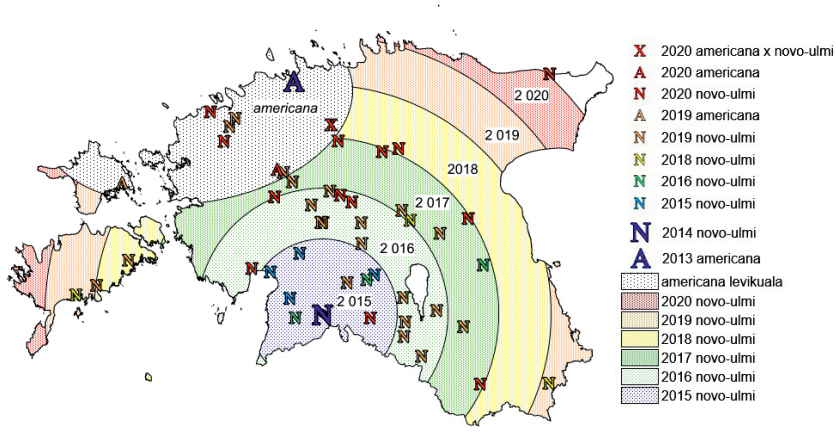


Joonis 5. Jalakasurma enamlevinud tekitaja (*O. novo-ulmi* subsp. *novo-ulmi*) nakkusega puude maksimaalne kaugus esimesest positiivsest proovikohast Tihemetsas 2014. aastal (L) ja leviku kiirus proovi kogumise kalendriaasta järgi.
Figure 5. Maximum distance of trees infected by DED pathogen (*O. novo-ulmi* subsp. *novo-ulmi*) from the first positive sampling site in Tihemetsa in 2014 (L) and rate of spread by calendar years of sample collections.

Jalakasurma tekitajate levikut Eestis on iseloomustatud patogeeni alamliigi järgi tehtud määrangute alusel (vt joonis 6).

Levikukaardilt (joonis 6) nähtuvad konkreetsete nakkusega puude leiukohad, millelt jalakasurm isoleeriti puhaskultuuri ja seejärel tuvastati täpselt molekulaarselt. Loode-Eestis on lisaks alamliigile *O. novo-ulmi* subsp. *novo-ulmi* levinud ka alamliik *O. novo-ulmi* subsp. *americana*, mille levikuala on joonisel 6 kujutatud mustatäpilise kontuurina. Olulisima tulemusena õnnestus kahe erineva alamliigi levikualade kattumise piirkonnas tuvastada agressiivsem jalakasurma tekitaja hübriid *O. novo-ulmi* subsp. *americana* x subsp. *novo-ulmi*. Täpsustavalt tuleb märkida, et antud levikukaart ei näita patogeeni varasemaid (enne 2013. aastat pärinevaid) leide (Hanso & Drenkhan, 2007; Drenkhan *et al.*, 2017), kuna varasemast ajast pärinevaid puhaskultuure pole säilinud ning täpne alamliik on teadmata. Seepärast näitavad tulemused (joonis 6) ainult patogeeni praeguse epideemia levikut (ja seda, mida suudeti isoleerida ja määrata). Niisamuti on võimalik, et patogeen võis levida laiali ka mitmest nakkuspunktist. Loodetavasti õnnestub patogeeni uus jalaka-siugsuu ja tema alamtaksonite

tegelikku algpäritolu ja levikutsentreid Eestis ja mujal tuvastada hilisemate seene populatsioonide geneetiliste uuringutega.



Joonis 6. Jalakasurma tekitajate positiivsete leiukohtade paiknemine („N“ – subsp. *novo-ulmi*, „A“ – subsp. *americana*) ning selle tulemusel hinnatud patogeeni levik kalendriaastate järgi kasutades regressioonanalüüsi. Patogeeni hübriid (X) *O. novo-ulmi* subsp. *americana* x *novo-ulmi* tuvastati Koselt, Põhja-Eestist.
Figure 6. Location of positive samples for DED pathogen subspecies ("N" – subsp. *novo-ulmi*, "A" – subsp. *americana*) and estimated spread of pathogen by calendar years using regression analysis. Pathogen hybrid (X) *O. novo-ulmi* subsp. *americana* x *novo-ulm*i was detected in Kose, North Estonia.

Jalakasurma peremeestaimed ja nende tervislik seisund

Klassi kaheidulehelised, roosilaadsete seltsi ja jalakaliste sugukonda kuuluvad euroopa jalakad kuuluvad kahte lahknevasse sektsiooni – *Blepharocarpus*, esindajaks künnapuu (*Ulmus laevis* Pallas) ja *Ulmus*, esindajateks harilik jalakas (*Ulmus glabra* Huds.) ja põldjalakas (*U. minor* Mill.) (Venturas *et al.*, 2014). Künnapuu teiste Euroopa kohalike jalaka liikidega ei hübridiseeru (Mittempergher & La Porta, 1991).

Eestis looduslikult kasvavad harilik jalakas ja künnapuu on vähelevinud metsapuud, moodustades *ca* 0,2% kogu metsatagavarast (Raudsaar *et al.*, 2018). Harilik jalakas on levinud kõikjal Eestis, kuid künnapuid võib leida pigem jõgede läheduses (Kukk & Kull, 2005). Künnapuu kuulub Eestis III kaitsekategooriasse (Lilleleht, 2008), mis tähendab, et ta on ohualdis. Mõlemast liigist on arvele võetud kõik olulisteks peetavad suured puud kogu Eestis (Relve, 2011).

Samas on jalakate perekonna esindajad sagedased haljastuspuud nii linnakeskkonnas (Aaspõllu, 1999; Kaar, 2011) kui ka maapiirkonnas, k.a ajaloolistes parkides (Abner *et al.*, 2007, 2012). Mõned neist ajaloolistest parkidest on kujundatud vanadest salumetsadest (Kalda, 1995; Tamm, 2007), mis on üks parimaid kasvukohti jalakatele (Paal, 1998). Haljastuses hinnatakse jalakaid eelkõige sellepärast, et nad taluvad keskkonnast tulenevat stressi, mida linnades tekitavad muu hulgas saastunud õhk, libedusetõrje soolad, tihenenud muld, kõikjal aga põuad ja ajutised üleujutused (Townsend & Douglass, 2004; Zalapa *et al.*, 2008).

Eestis on üldiselt künnapuude tervislik seisund parem kui harilikul jalakal, mida kinnitab ka 2014–2016 aastatel tehtud võrdlev puude tervisliku seisundi analüüs (Jürisoo *et al.*, 2019). Sama tulemus on saadud ka Loode-Venemaal Peterburis (Jürisoo *et al*., 2021) ja Poolas (Łakomy *et al.*, 2016). Sama võib kaudselt kinnitada ka see, et iga üheteistkümnes harilik jalakas oli nakatunud jalakasurmaga, künnapuude puhul oli jalakasurmaga nakatunud vaid iga 37. puu (tabel 3). Künnapuudel võib olla parem tervis tänu sellele, et nad on vähem atraktiivsed jalakasurma tekitajat levitavatele vektorputukatele (Santini & Faccoli, 2013).

Tabel 3. Eestis hinnatud puude, korjatud haigusproovide ja puhaskultuuri isoleeritud jalakasurma tekitajate juhtumite arv aastate lõikes
Table 3. *Number of assessed trees, collected samples and isolates of DED agent in different years in Estonia*

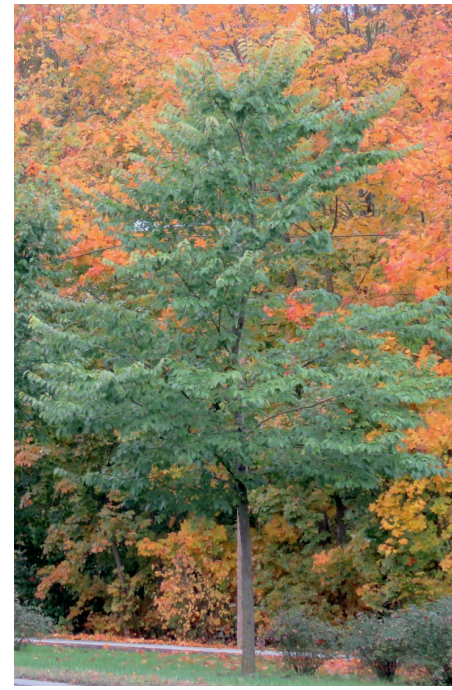
	Aasta						Kokku
	2014	2015	2016	2018	2019	2020	
Hinnatud puid	158	313	759	107	211	672	2220
sh harilik jalakas	147	286	587	100	189	614	1923
sh künnapuu	9	26	147	0	17	58	257
Kogutud proove	42	91	105	22	182	76	518
sh harilik jalakas	42	80	93	22	169	75	481
sh künnapuu	0	10	5	0	13	1	29
Jalakasurma tekitaja puhaskultuuris	1	38	36	9	54	45	183
sh harilikul jalakal	1	36	34	9	52	44	176
sh künnapuul	0	2	2	0	2	1	7

Peremeestaimede hukkumine jalakasurma tõttu sõltub jalaka liigist (*Ulmus*), konkreetse puu vastuvõtlikkusest (Martín et al., 2019b), puistu tihedusest ja võimalikest juurekontaktidest haigete ja tervete puude vahel (Santini & Faccoli, 2013).

Jalakasurmale resistentsete jalakate sordiaretust alustati kohe pärast jalakasurma avastamist. Seda on tehtud Hollandis, Itaalias, Hispaanias ja Põhja-Ameerikas (Pajares *et al.*, 2004) ning enamasti on selleks ristatud kohalikke liike Aasia liikidega, mis on jalakasurmale vastupidavamad (Zalapa *et al.*, 2008).

Resistentseid jalakaid ‘New Horizon’ (Johannes Grothaus, saksa puukooli Lorberg maastikuarhitekt, Euroroute R1 korraldaja, suulised andmed), mis on Jaapani ja madala jalaka hübriid (*Ulmus davidiana* var. *japonica* × *U. pumila*), istutati Eesti linnadesse (nt Tartu, Valga, Narva) 2015. aastal. Järgmisel aastal oli aga neil enamik viimase aasta võrsetest külmakahjustusega (joonis 7). Viimastel aastatel on need jalakad siiski olnud terved (joonis 8) ja seda ilmselt pehmete talvede tõttu. Siiski on nii lühikese ajaperioodi vältel vara hinnata nende hübriidjalakate tegelikku vastupidavust meie keskkonnatingimustele.

Joonis 7. Hübriidjalakas ‘New Horizon’ Narvas 2016. a
Figure 7. Hybrid elm cultivar ‘New Horizon’ in Narva in 2016.



Joonis 8. Hübriidjalakas ‘New Horizon’ Tartus 2019. a
Figure 8. Hybrid elm ‘New Horizon’ in Tartu in 2019

Euroopas resistentsetena tuntud jalakasortide kasutamine on üks võimalus jalakate säilimiseks haljastuses ka jalakasurma keskkonnas, kuid mõistlik oleks enne haljastuses kasutamist nende sobivust testida kohalikes, sh Eesti tingimustes. Seda just sellepärast, et näiteks Peterburi istutatud nn potentsiaalselt resistentseid hübriidjalakaid kahjustas jalakasurm niisamuti nagu

kohalikke jalakaliike ja osad neist tuli üsna varsti pärast istutamist välja raiuda (Jürisoo *et al.*, 2021).

Lisaks tuleb otsida haigusele vähem vastuvõtlikke isendeid ja paljundada nende järglasi ka kohalike liikide hulgast (Martín *et al.*, 2009). Oleme selle suunitlusega rajanud jalakaliikide järglaskatsealasid Järvseljale ja ka mujale Eestis. Katsealadele on istutatud erinevatest asukohtadest korjatud hariliku jalaka ja künnapuu ühe kindla puu järglased, samuti kaks erinevat perspektiivikat hübriidjalakat 'New Horizon' (*U. japonica* x *U. pumila*) ja 'Fiorente' (*U. pumila* x *U. minor*).

Kõigele lisaks tuleks linnades eelistada puittaimede liigilist mitmekesisust (Haugen, 1998). Näiteks Lõuna-Rootsis Malmös otsustati pärast enamuse nakatunud jalakate raiet, et eesmärgiks oleks kasvatada linnas kuni 500 erinevat puu taksonit ja pikemas perspektiivis veel enamgi (Arne Mattsson, suulised andmed). Hetkel on Malmös jalakad asendatud teiste, nii kodumaiste kui ka introdutseeritud liikidega, saades seejärel teiste seas suurima liigirikkusega linnaks Skandinaavias (Sjöman *et al.*, 2012).

Jalakasurma putukavektorid ja nende tuvastamine

On arvatud isegi, et Põhja-Euroopas pole jalakasurma levitavaid putukaid ja selle tõttu pole ka haigust (La Porta *et al.*, 2008). Tõepoolest ei ole Soomest sobivaid vektorputukaid leitud (Voolma *et al.*, 2004; Hannunen & Marinova-Todorova, 2016). Eestis on neid putukaid täheldatud juba möödunud sajandi esimeses pooles (Voolma *et al.*, 2004, 2000) – vähesel määral suur-maltsaüraskit (*S. scolytus*), läikivat maltsaüraskit (*S. laevis*), väike-maltsaüraskit (*S. multistriatus*) ja viimasel ajal ka jalaka-maltsaüraskit (*S. triarmatus*) (Süda, 2006). Seega ei ole küsimus haiguse levitajates, vaid pigem kliima soojenemises ja globaalses kaubanduses (Drenkhan *et al.*, 2017a). Temperatuur mõjutab nii maltsaüraskite biogeograafiat ja levikut kui ka muid olulisi tegureid, nagu kevadise lennu algust, arengu kiirust, põlvkondade arvu aastas ja suremust (eelkõige talvel). Sooja suvega võib kahjuril areneda kasvuperioodi jooksul 2-3 põlvkonda (Heliövaara & Peltonen, 1999) ning talvituvad nii vastsed kui ka valmikud. Valmikute küpsussööm on juunis-juulis, mõnedel ka alles augustis-septembris (Beaver, 1969). Putukate lendlusaeg Rootsis toimub maist septembrini (Anderbrant & Schlyter, 1987), kuid selle maksimum on juulis-augustis (Menkis *et al.*, 2016). Näiteks kattus Eestis 2019. aastal putukate lendlusaeg Rootsis registreeritud andmetega (Jürisoo *et al.*, 2021).

Haiguse esmane levik tervele puule toimub jalaka maltsaüraskite küpsussööma ajal, kus noormardikas närib tavaliselt oksa harunemiskohal kambiumisse kuni 1 cm käigu (Baker &

Norris, 1968; Pajares, 2004; Rabaglia & Lanier, 1983; Süda, 2006). Maltsaüraskite massiline rünnak toimub pigem stressis puule. Kuna jalakasurma sümptomid puul on näha alles selle hilises faasis, oleks parim, kui nakatunud puud langetatakse ja hävitatakse enne üraskite küpsussööma (Baker & Norris, 1968; Rabaglia & Lanier, 1983).

Putukad eelistavad mõningaid jalaka liike teistele või isegi liigisiseselt mõningaid isendeid teistele. Üldiselt on harilik jalakas ja künnapuu vähem atraktiivsed maltsaüraskitele kui põldjalakas ja madal jalakas (*U. pumila* L.), seda puu koores esinevate teatud keemiliste ühendite tõttu (Pajares, 2004; Webber, 2004). Niisamuti on künnapuu hariliku jalakaga võrreldes putukatele vähem atraktiivne (Santini & Faccoli, 2013). Samuti on leitud, et putukatele resistentsemad isendid võivad olla ka haigusele resistentsemad (Baker & Norris, 1968; Rabaglia & Lanier, 1983). Kohalike puude resistentsust aga saab ja tuleb testida nakatamiskatsega.

Jalakasurma levikul ja tõrjeabinõude rakendamisel on oluline teada kohapeal esinevate võimalike vektorliikide arvukust (Jürisoo et al., 2021b). Vektorputukate avastamiseks ja nende arvukuse hindamiseks kasutatakse feromoonpüüniseid. Feromoonid on teatavasti putukate poolt toodetavad lenduvad keemilised ühendid, mis mõjutavad putukate käitumist, näiteks paaritumisel või peremeestaime äratundmisel ning need ühendid on väga liigispetsiifilised (Vanatoa, 2004).

Kanada firma Synergy Semiochemicals on ainus, kes toodab spetsiaalseid maltsaüraskitele mõeldud feromoone, mida kasutatakse peibutusena ja mis aitavad jälgida maltsaüraskite populatsioone, nende olemasolu, lennuperioodi jms. Feromoonpüünised pole kuigi efektiivsed putukate massiliseks püüdmiseks (El-Sayed *et al.*, 2009; Haugen, 1998) ega ka paaritumise takistamiseks (El-Sayed *et al.*, 2006). Feromoone võib pigem kasutada suurte putukahulkade ligimeelitamiseks, et need ründaksid püünispuid, et siis need puud langetada ja hävitada. Tuleb aga arvestada, et feromoonidega meelitatakse putukad püünisesse, kuid iga 50 meetri raadiuses olev jalakas on potentsiaalne sihtmärk vektorputukatele (Synergy Semiochemicals Corporation, 2019), mille tagajärjel võib jalakasurma levik veelgi intensiivistuda (Boutz *et al.*, 2009).

Kuigi patogeeni ja vektori kooseksisteerimine on pigem juhuslik, siis on nad tihedalt omavahel seotud: maltsaüraskid on ainsaks võimalikuks looduslikuks haigusetekitaja levitajaks pikemate vahemaade taha ja seega oluliseks lüliks epideemia arengus. Vastutasuks haiguse levitamisele on putukatel rohkem süüa, sest nad toituvad eelkõige just stressis olevate ja surevate puude värskest maltspuidust, selle tõttu suureneb nende arvukus ja tulemuseks levib haigus kiiremini.

Kui üks nendest lülidest kaob, siis haiguse levik ja intensiivsus aeglustub (Baker & Norris, 1968; Rabaglia & Lanier, 1983).

Jalakasurma tõrje võimalused

Jalakasurma tõrje on keeruline ja selleks kasutatud erinevad meetmed pole tihti andnud soovitud tulemusi (Pecori *et al.*, 2017).

Keemilisi tõrjevahendeid jalakasurma vastu on uuritud alates 1930ndatest, läbi on proovitud rohkem kui 600 erinevat preparaati nii mulda viies kui ka puu tüvesse süstides (Stipes, 2000). Süsteemseid fungitsiide on kasutatud nn vaktsiinidena tervete või üksikute sümptomitega puude kaitsmiseks (Scheffer *et al.*, 1988; Stipes, 2000), kuid kasutusse on neist jäänud vaid üksikud, näiteks Arbotect-20® ja Alamo (Stennes, 2000). Keemilised tõrjevahendid metsas ja linnakeskkonnas pole aga paljudes riikides lubatud, k.a Eestis. Samas olulist pikaajalist tõrje-efektiivsust need preparaadid paraku ei pakugi.

Jalakasurma biotõrje otsingutel on uuritud baktereid, seeni ja mükoviiruseid. Bakterite *Pseudomonas* spp. süstimine jalakatesse on mingil määral aidanud vähendada *O. novo-ulmi* kasvukiirust puus (Myers & Strobel, 1983) või siis tõstnud peremeestaime resistentsust haiguse suhtes (Scheffer, 1983), kuid seegi meetod pole osutunud piisavalt efektiivseks (Stipes, 2000). Lootustandvamatest meetoditest tervete jalakate vaktsineerimine seeneliigi *Verticillium dahliae* eosmassiga on aidanud tõsta jalakate resistentsust *O. novo-ulmi* suhtes (Scheffer, 1990; Elgersma *et al.*, 1993) ning seda biotõrje meetodit kasutatakse siiani Hollandis, Saksamaal, Rootsis, Kanadas ja USAs (Voeten *et al.*, 2009; Postma *et al.*, 2014). See meetod on aga kallis, kuna vaktsineerimist tuleb igal kevadel korrata ja olenemata sellest haigestub vähene hulk jalakatest ikkagi. Seega saab puude vaktsineerimine (Voeten *et al.*, 2009) toimida vaid väga väärtuslike puude kaitseks, näiteks ajaloolistes parkides. Vaktsineerimine saab siiski olla vaid üks osa integreeritud taimekaitsest (Postma *et al.*, 2014). Jalakasurma on püütud tõrjuda veel teistegi antagonistlike seentega (nt *Monographella nivalis*, *Alternaria tennuissima*), kuid häid tulemusi pole need andnud (Hubbes & Jeng, 1981; Sutherland *et al.*, 1995; Blumenstein, 2015). On teada, et mükoviirused aitavad oluliselt vähendada jalakasurma tekitaja *O. novo-ulmi* patogeensust (Webber, 1987; Swinton & Gilligan, 1999). Seda on kasutatud USAs (Brasier, 2000), kuid puuduseks on asjaolu, et need mõjuvad pärssivalt ainult teatud patogeeni tüvedele ja seetõttu ei ole meetod piisavalt universaalse toimega ega laialt kasutatav (Ganley & Bulman, 2016).

Seega ei ole senised keemilised ja ka biotõrje meetodid viinud olulise eduni jalakasurma tõrjel. Siiski otsivad teadlased jätkuvalt võimalikke mikroorganisme, kelle abil saaks efektiivsemalt rakendada biotõrjet jalakasurma vastu (Blumenstein, 2015; Pepori *et al.*, 2018).

Soovitusi ja põhimõtteid jalakasurma tõrjel

Praeguste teadmiste kohaselt on kõige efektiivsemaks tõrjeks haigete puude kiire avastamine ja patogeeni määramine ehk süsteemne monitooring ja nende õigeaegne raie. Selleks tuleb jalakaid hinnata vähemalt üks kord aastas, eelistatult alates juulikuust ja seda nii linnades kui ka maapiirkondades (Stipes & Campana, 1981; Arne Mattsson, Malmö endine linnaaednik, suulised andmed). Seni on inventeerimist süsteemsemalt tehtud vaid Tallinnas ja Tartus, kuid ka seal puudub ettekujutus puude liigilisest koosseisust eramaadel. Süsteemne puude inventeerimine on näiteks Malmös aidanud kaasa jalakasurma ja sellele vastuvõtlike isendite kiirele tuvastamisele ja seega ka haiguse leviku aeglustumisele (Morgenroth & Östberg, 2017). Jalakasurma ohu vähendamisele aitaks kaasa ka vabakaubanduse sihikindel reguleerimine seadusandluse ja kontrollisüsteemi abil, mis tagaks, et me ei tooks riiki uusi patogeene juhusliku ja teadmata päritolu taimse materjali kaudu (Drenkhan *et al.*, 2017a; Roy *et al.*, 2014), lisaks peavad istikud vastama istikute kvaliteedinõuetele (EVS 939-2, 2020).
Avastatud haiged puud tuleb kasvuperioodil eemaldada hiljemalt 2-3 nädala jooksul nende avastamisest (Haugen, 1998) või siis puude puhkeperioodil enne aprillikuud. Raiutud nakatunud jalakate puit tuleb transportida esimesel võimalusel kinnistes konteinerites (Arne Mattsson, suulised andmed), sest vastasel juhul ei saa ära hoida vektorputukate levikut. Kui puitu ei ole võimalk transportida, siis tuleb langetatud puudel eemaldada koor (Liberato *et al.*, 2016) või peenestada hakkeks. Eelistatult soovitame ka nakatunud puude oksad ja puit hakkida või oksad kohapeal põletada.

Kokkuvõte ja järeldused

Jalakasurm on Eesti jalakaid kahjustanud teadaolevalt juba alates eelmise sajandi 30-ndatest aastatest. Esmalt oli patogeeniks *Ophiostoma ulmi* (ee k jalaka-siugsuu), mis nüüdseks on asendunud uue liigiga *Ophiostoma novo-ulmi*: uus jalaka-siugsuu.
Eestis on jalakasurma seiret ja jalakasurma tekitajaid detailsemalt uuritud alates 2014. aastast. Perioodil 2014–2020 on hinnatud üle 2200 jalakate perekonna esindaja, korjatud enam kui 500 haigusproovi, millest isoleeriti patogeeni puhaskultuurid. Seene isolaate on analüüsitud mitme

molekulaarse praimeriga, mille tulemusel on tuvastatud haigustekitaja põhiliik (*Ophiostoma novo-ulmi*) ning selle alamliigid. Enamasti levib Eestis jalakasurma tekitaja Euroopa alamliik (subsp. *novo-ulmi*) ja vähem Põhja-Ameerika alamliik (subsp. *americana*), viimane vaid Põhja-Eestis ja Hiiumaal. Esimest korda tõestati ka patogeeni agressiivse hübriidi esinemine Eestis, seda on esialgu tuvastatud vaid Kosel Harjumaal patogeeni kahe alamliigi levikupiiril. Uurimistöö põhjal leidis kinnitust jalakasurma tekitajate esinemine Eesti 14 maakonnas, sh Tallinna, Tartu, Pärnu ja Viljandi linnas. Jalakasurma ei ole leitud Lääne-Viru maakonnas.

Kohalikest peremeestaimedest on haigusele vastuvõtlikum harilik jalakas, künnapuu tervislik seisund on oluliselt parem ja ka jalakasurma tekitajat üldse on leitud vaid üksikutelt künnapuudelt (vt tabel 3). Seepärast soovitame haljastuses kasutada jalakalistest pigem künnapuud. Haljasaladel võivad olla perspektiivikad resistentsemad jalakate sordid, kuid need vajavad enne laialdast kasutusele võtmist veel testimist.

Jalakasurma tekitaja peamisteks levitajateks on jalaka-maltsaüraskid (*Scolytus* spp.) ja eelkõige soodustab nende putukate levikut kliima soojenemine.

Teadlased otsivad pingsalt võimalusi, kuidas jalakasurma efektiivsemalt tõrjuda, kuid hästi töötavaid universaalseid tõrjevõimalusi pole seni õnnestunud leida. Kui haigus avastatakse algstaadiumis ja esineb alles üksikutel puudel, siis aitab haigete puude kiire kõrvaldamine patogeeni levikut oluliselt pidurdada.

Summary

The threat to *Ulmus* spp. has risen in Estonia, apparently due to the trade of infected elm plants and changing climatic conditions having contributed to the invasion of new pathogens and their vectors. It is known that in Estonia Dutch Elm Disease (DED) has had a devastating impact on elms since the 1930s. Today its agent *Ophiostoma ulmi* has been replaced by a new, apparently an invasive species *Ophiostoma novo-ulmi* in most countries of Europe, as well as in Estonia. Since 2013 the health status of elms in Estonia has worsened substantially.

The aim of this research was to analyse the current spread of DED until 2020, specify the taxonomy of pathogens, evaluate the health status of host plants, and analyse the control options of the disease.

Visual assessment has been provided for the period of 2014 to 2020. Methods and results for the years 2014–2016 are presented in a previous paper (see Jürisoo *et al.*, 2019).

More precise monitoring of Dutch elm disease in Estonia started in 2014. A preliminary map of DED was provided on the basis of the assessments of the distribution (records). Thereafter, in the period of 2014–2020 more than 2,200 trees of genus *Ulmus*, mostly *Ulmus glabra* were assessed. Also, over 500 samples were collected of which 183 pure cultures of *Ophiostoma novo-ulmi* were isolated and analysed with different molecular primers (Table 3). In the period of 2018–2020, DED was detected in 14 counties of the total of 15 counties in Estonia. The analyses show that *O. novo-ulmi* subsp. *novo-ulmi* is widely spread; however, *O. novo-ulmi* subsp. *americana* was detected only in northern Estonia and on Hiiumaa island in western Estonia (Fig. 6). For the first time the hybrid pathogen (*americana* x *novo-ulmi*) was detected at a sampling site at Kose in northern Estonia (Figure 6).

Our investigation demonstrated that in terms of the use of *Ulmus* as an amenity tree species *Ulmus laevis* could be considered more prospective than *U. glabra* because the health of *U. laevis* is significantly better and DED is not so devastating to the host. Also, we have to test resistant hybrid elms in our environmental conditions before starting massive planting in our green areas. All the plant material should meet the local standard on Quality Requirements for the Nursery Plants (see EVS 939-2, 2020). We have created several provenance trials in Estonia to qualify tolerant elm species or origins in the future.

DED control is complicated because effective universal control methods are not available. Thus, a good sanitation program together with a reliable survey is needed. Symptomatic elms should be felled and completely destroyed; this seems to be the most effective method for DED control. Introduced planting material should be certified and controlled to minimise the invasion of diseases and pests.

Tänusõnad

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Kasutatud kirjandus

Aaspõllu, A., 1999. Lehtpuude kultivarid Eestis, Sander, H. (Toim.), Dendroloogilised Uurimused Eestis. lk. 110–137.

Aaspõllu, A., 2017. Kodumaiste lehtpuuliikide olukord järjest halveneb. Maaelu.

Abner, O., Konsa, S., Lootus, K., Sinijärv, U., 2007. Eesti pargid 1. Keskkonnaministeerium, Muinsuskaitseamet, Varrak, Tallinn. 423 lk.

Abner, O., Konsa, S., Lootus, K., Sinijärv, U., 2012. Eesti pargid 2. Keskkonnaministeerium, Muinsuskaitseamet, Varrak, Tallinn. 647 lk.

Anderbrant, O., Schlyter, F., 1987. Ecology of the Dutch Elm Disease Vectors Scolytus laevis and S. scolytus (Coleoptera: Scolytidae) in Southern Sweden. J. Appl. Ecol. 24, 539. https://doi.org/10.2307/2403891

Aotäht, A., 2018. Linn loodab jalakasurma uurijale. Viljandi Sakala 1.

Baker, J.E., Norris, D.M., 1968. Further Biological and Chemical Aspects of Host Selection by *Scolytus multistriatus*. Ann. Entomol. Soc. Am. 61, 1248–1255. https://doi.org/10.1093/aesa/61.5.1248

Beaver, R.A., 1969. Natality mortality and control of the elm bark beetle *Scolytus scolytus* Coleoptera Scolytidae. Bull. Entomol. Res. 59, 537–540.

Blumenstein, K., 2015. Endophytic fungi in elms: implications for the integrated management of Dutch elm disease. Southern Swedish Forest Research Centre, Swedish University of Agricultural Sciences. 84 lk.

Boutz, G.E., Brewer, J.W., Bishop, J.N., 2009. Capture patterns of *Scolytus multistriatus* (Marsh.) (Col., Scolytidae) attracted to a pheromone-baited trap. Zeitschrift für Angew. Entomol. 99, 366–370. https://doi.org/10.1111/j.1439-0418.1985.tb01999.x

Brasier, C.M., 1979. Dual origin of recent Dutch elm disease outbreaks in Europe. Nature 281, 78–80. https://doi.org/10.1038/281078a0

Brasier, C.M., 1990. China and the origins of Dutch elm disease: an appraisal. Plant Pathol. 39, 5–16. https://doi.org/10.1111/j.1365-3059.1990.tb02470.x

Brasier, C.M., 1991. *Ophiostoma novo-ulmi* sp. nov., causative agent of current Dutch elm disease pandemics. Mycopathologia 115, 151–161. https://doi.org/10.1007/BF00462219

Brasier, C.M., 2000a. Intercontinental Spread and Continuing Evolution of the Dutch Elm Disease Pathogens, Dunn, C.P. (Toim.), The Elms: Breeding, Conservation, and Disease Management. Springer US, lk. 61–72. https://doi.org/10.1007/978-1-4615-4507-1_4

Brasier, C.M., 2000b. Viruses as biological control agents of the Dutch elm disease fungus Ophiostoma novo-ulmi, in: Dunn, C.P. (Toim.), The Elms - Breeding, Conservation and Disease Management. Kluwer Academic Publishers, Boston, USA, lk. 201–212.

Brasier, C.M., 2001. Rapid Evolution of Introduced Plant Pathogens via Interspecific Hybridization is

leading to rapid evolution of Dutch elm disease and other fungal plant pathogens. Bioscience 51, 123–133. https://doi.org/10.1641/0006-3568(2001)051[0123:reoipp]2.0.co;2

Brasier, C.M., 2008. The biosecurity threat to the UK and global environment from international trade in plants. Plant Pathol. 57, 792–808. https://doi.org/10.1111/j.1365-3059.2008.01886.x

Brasier, C.M., Buck, K.W., 2001. Rapid Evolutionary Changes in a Globally Invading Fungal Pathogen (Dutch Elm Disease). Biol. Invasions 3, 223–233. https://doi.org/10.1023/A:1015248819864

Brasier, C.M., Kirk, S.A., 2000. Survival of clones of NAN *Ophiostoma novo-ulmi* around its probable centre of appearance in North America. Mycol. Res. 104, 1322–1332. https://doi.org/10.1017/S0953756200002732

Brasier, C.M., Kirk, S.A., 2001. Designation of the EAN and NAN races of *Ophiostoma novo-ulmi* as subspecies. Mycol. Res. 105, 547–554. https://doi.org/10.1017/S0953756201004087

Brasier, C.M., Kirk, S.A., 2010. Rapid emergence of hybrids between the two subspecies of *Ophiostoma novo-ulmi* with a high level of pathogenic fitness. Plant Pathol. 59, 186–199. https://doi.org/10.1111/j.1365-3059.2009.02157.x

Brasier, C.M., Mehrotra, M.D., 1995. *Ophiostoma himal-ulmi* sp. nov., a new species of Dutch elm disease fungus endemic to the Himalayas. Mycol. Res. 99, 205–215. https://doi.org/10.1016/S0953-7562(09)80887-3

Brasier, C.M., Webber, J.F., 2019. Is there evidence for post-epidemic attenuation in the Dutch elm disease pathogen *Ophiostoma novo-ulmi*? Plant Pathol. 68, 921–929. https://doi.org/10.1111/ppa.13022

Caulton, E., Aitken, W., Rashid, N., 1998. Aerobiological aspects of elm (*Ulmus* spp.) in South-East Scotland in relation to elm decline from Dutch elm disease (1976–1996). Aerobiologia (Bologna). 14, 147–153. https://doi.org/10.1007/BF02694199

Clinton, G.P., McCormick, F.A., 1936. Dutch elm disease. *Graphium ulmi*. New Haven. lk. 701-752.

Corfixen, P., Parmasto, E., 2005. *Hymenochaete ulmicola* sp. nov. (Hymenochaetales). Mycotaxon 91, 465–469.

Drenkhan, R., Adamson, K., Drenkhan, T., Agan, A., Laas, M., 2017a. Uus probleemistik dendropatoloogias – uued ja invasiivsed patogeenid. Metsanduslikud Uurim. 67, 50–71.

Drenkhan, R., Solheim, H., Bogacheva, A., Riit, T., Adamson, K., Drenkhan, T., Maaten, T., Hietala, A.M., 2017b. Hymenoscyphus fraxineus is a leaf pathogen of local *Fraxinus* species in the Russian Far East. Plant Pathol. 66, 490–500. https://doi.org/10.1111/ppa.12588

Dvořák, M., Tomšovský, M., Jankovský, L., Novotný, D., 2007. Contribution to identify the causal agents of Dutch elm disease in the Czech Republic. Plant Prot. Sci. 43, 142–145.

eElurikkus [WWW dokument], 2019. *Ophiostoma ulmi*. URL https://elurikkus.ee/bie-hub/species/161882#classification (vaadatud 14.12.19).

El-sayed, A.M., Suckling, D.M., Byers, J.A., Jang, E.B., Wearing, C.H., Econ Entomol, J., 2009. Potential of 'Lure and Kill' in Long-Term Pest Management and Eradication of Invasive Species.

J. Econ. Entomol. 102, 815–835.

El-Sayed, A.M., Suckling, D.M., Wearing, C.H., Byers, J.A., 2006. Potential of Mass Trapping for Long-Term Pest Management and Eradication of Invasive Species. J. Econ. Entomol. 99, 1550–1564. https://doi.org/10.1093/jee/99.5.1550

Elgersma, D.M., Roosien, T., Scheffer, R.J., 1993. Biological Control of Dutch Elm Disease by Exploiting Resistance in the Host, in: Sticklen, M.B., Sherald, J.L. (Eds.), Dutch Elm Disease Research. Springer New York, New York, NY, pp. 188–192. https://doi.org/10.1007/978-1-4615-6872-8_15

Ellstrand, N.C., Schierenbeck, K.A., 2000. Hybridization as a stimulus for the evolution of invasiveness in plants? Proc. Natl. Acad. Sci. U. S. A. 97, 7043–50. https://doi.org/10.1073/pnas.97.13.7043

EPPO Global Database [WWW dokument], 2019. EPPO. URL https://gd.eppo.int/ (vaadatud 07.06.19).

EVS 939-2, 2020. PUITTAIMED HALJASTUSES Osa 2: Ilupuude ja -põõsaste istikute kvaliteedinõuded, 26 lk. Eesti Standardimis- ja Akrediteerimiskeskus. 28 lk.

Ganley, R.J., Bulman, L.S., 2016. Dutch elm disease in New Zealand: impacts from eradication and management programmes. Plant Pathol. 65, 1–9. https://doi.org/10.1111/ppa.12527

Ghelardini, L., Luchi, N., Pecori, F., Pepori, A.L., Danti, R., Della Rocca, G., Capretti, P., Tsopelas, P., Santini, A., 2017. Ecology of invasive forest pathogens. Biol. Invasions 19, 3183–3200. https://doi.org/10.1007/s10530-017-1487-0

Gibbs, J.N., 1978. Intercontinental Epidemiology of Dutch Elm Disease. Annu. Rev. Phytopathol. 16, 287–307. https://doi.org/10.1146/annurev.py.16.090178.001443

Gibbs, J.N., Brasier, C.M., 1973. Correlation between cultural characters and pathogenicity in *Ceratocystis ulmi* from Britain, Europe and America. Nature 241, 381–383. https://doi.org/10.1038/241381a0

Hallin, A.-K., 2010. Ash and elm join the Swedish Red List [WWW dokument]. Biowissenschaften Chemie. URL https://www.innovations-report.de/html/berichte/biowissenschaften-chemie/ash-elm-join-swedish-red-list-153585.html (vaadatud 16.06.20).

Hannunen, S., Marinova-Todorova, M., 2016. Pest Risk Assessment for Dutch elm disease. Helsinki. 110 lk.

Hanso, M., Drenkhan, R., 2007. Metsa- ja linnapuud ilmastiku äärmuste vaevas. Eesti Loodus 58, 6–13.

Hantula, J., 2021. The story of Dutch elm disease (Hollaninjalavataudi tarina). Sorbifolia 52, 31–35. [Soome keeles]

Haugen, L., 1998. How to Identify and Manage Dutch Elm Disease. St. Paul, MN, USA.

Heliövaara, K., Peltonen, M., 1999. Bark Beetles in a Changing Environment. Ecol. Bull. 47, 48–53. https://doi.org/10.2307/20113226

Herald, 2019. [WWW dokument]. Hundreds of Dutch elm disease trees to be felled. Her. Scotl. Online. https://www.heraldscotland.com/news/17535477.hundreds-dutch-elm-disease-trees-felled/

(vaadatud 26.10.20).

Hintikka, V., 1974. *Ceratocystis ulmi* in Finland. Karstenia 14, 32–32. https://doi.org/10.29203/ka.1974.87

Hubbes, M., Jeng, R.S., 1981. Aggressiveness of *Ceratocystis ulmi* strains and induction of resistance in *Ulmus americana*. For. Pathol. 11, 257–264. https://doi.org/10.1111/j.1439-0329.1981.tb00094.x

Jüriado, I., Liira, J., Paal, J., 2009. Diversity of epiphytic lichens in boreo-nemoral forests on the North-Estonian limestone escarpment: The effect of tree level factors and local environmental conditions. Lichenologist 41, 81–96. https://doi.org/10.1017/S0024282909007889

Jürisoo, L., 2015a. Voltveti mõisa pargi hoolduskava 2015-2024. 85 lk.

Jürisoo, L., 2015b. Heimtali pargi dendroloogiline hinnang. 30 lk.

Jürisoo, L., Adamson, K., Padari, A., Drenkhan, R., 2019. Health of elms and Dutch elm disease in Estonia. Eur. J. Plant Pathol. 154, 823–841. https://doi.org/10.1007/s10658-019-01707-0

Jürisoo, L., Selikhovkin, A. V., Padari, A., Shevchenko, S. V., Shcherbakova, L.N., Popovichev, B.G., Drenkhan, R., 2021a. The extensive damage to elms by Dutch elm disease agents and their hybrids in northwestern Russia. Urban For. Urban Green. 63, 127214. https://doi.org/10.1016/j.ufug.2021.127214

Jürisoo, L., Süda, I., Agan, A., Drenkhan, R., 2021b. Vectors of Dutch Elm Disease in Northern Europe. Insects 12, 393. https://doi.org/10.3390/insects12050393

Kaar, E., 2011. Jalakas ja künnapuu Eestis. Eesti Loodus 62.

Kalamees, K., 2011. Roosa võrkheinik: seenharuldus jalakal. Eesti Loodus 62, 41–41.

Kalamees, K., Sõmermaa, A.-L., 2000. Selts Siugsuudmelaadsed, Ophiostomatales, in: Kalamees, K. (Toim.), Eesti Seenestik. EPMÜ ZBI, Tartu, lk. 52.

Kalda, A., 1995. Broadleaved forests in Estonia, in: Aaviksoo, K., Kull, K., Paal, J., Trass, H. (Eds.), Consortium Masingii. pp. 89–95.

Konrad, H., Kirisits, T., Riegler, M., Halmschlager, E., Stauffer, C., 2002. Genetic evidence for natural hybridization between the Dutch elm disease pathogens *Ophiostoma novo-ulmi* ssp. *novo-ulmi* and *O. novo-ulmi* ssp. *americana*. Plant Pathol. 51, 78–84.

Kukk, T., Kull, T., 2005. Eesti taimede levikuatlas. Eesti Maaülikooli Põllumajandus- ja Keskkonnainstituut, Tartu. 528 lk.

La Porta, N., Capretti, P., Thomsen, I.M., Kasanen, R., Hietala, A.M., Von Weissenberg, K., 2008. Forest pathogens with higher damage potential due to climate change in Europe. Can. J. Plant Pathol. 30, 177–195. https://doi.org/10.1080/07060661.2008.10540534

Laas, E., Treumuth, S., 2006. Voltveti mõisapargi ja Tihemetsa arboreetumi dendroloogiline inventuur. Tartu. 78 lk.

Łakomy, P., Kwaśna, H., Kuźmiński, R., Napierała-Filipiak, A., Filipiak, M., Behnke, K., Behnke-Borowczyk, J., 2016. Investigation of *Ophiostoma* population infected elms in Poland.

Dendrobiology 76, 137–144. https://doi.org/10.12657/denbio.076.013

Lepik, E., 1940a. Estonia. The Elm disease in the country. Int. Bull. Plant Prot. 14, 2.

Lepik, E., 1940b. Miks kuivavad meie jalakad? Postimees 160, 5.

Liberato, J.R., Scott, C.R., Dick, M.A., Inglis, C., Dunnell, K.L., Bergdahl, A.D., 2016. Dutch Elm Disease, in: Diseases of Trees in the Great Plains. USDA Forest Service, pp. 126–128.

Lilleleht, V., 2008. Punane Raamat. Eesti Teaduste Akadeemia Looduskaitse Komisjon, Tartu. 150 lk.

Martín, J.A., Sobrino-Plata, J., Coira, B., Medel, D., Collada, C., Gil, L., 2019. Growth resilience and oxidative burst control as tolerance factors to Ophiostoma novo-ulmi in *Ulmus minor*. Tree Physiol. 39, 1512–1524. https://doi.org/10.1093/treephys/tpz067

Martín, J.A., Sobrino-Plata, J., Rodríguez-Calcerrada, J., Collada, C., Gil, L., 2019. Breeding and scientific advances in the fight against Dutch elm disease: Will they allow the use of elms in forest restoration? New For. 50, 183–215. https://doi.org/10.1007/s11056-018-9640-x

Martín, J.A., Solla, A., Esteban, L.G., de Palacios, P., Gil, L., 2009. Bordered pit and ray morphology involvement in elm resistance to *Ophiostoma novo-ulmi*. Can. J. For. Res. 39, 420–429. https://doi.org/10.1139/X08-183

Matisone, I., Kenigsvalde, K., Zaļuma, A., Burņeviča, N., Šņepste, I., Matisons, R., Gaitnieks, T., 2020. First report on the Dutch elm disease pathogen *Ophiostoma novo-ulmi* from Latvia. For. Pathol. e12601. https://doi.org/10.1111/efp.12601

Menkis, A., Östbrant, I.L., Davydenko, K., Bakys, R., Balalaikins, M., Vasaitis, R., 2016. *Scolytus multistriatus* associated with Dutch elm disease on the island of Gotland: phenology and communities of vectored fungi. Mycol. Prog. 15, 1–8. https://doi.org/10.1007/s11557-016-1199-3

Mihkelson, L., 2017. Seenhaigus tapab jalakaid. Maaleht.

Mittempergher, L., La Porta, N., 1991. Hybridization studies in the Eurasian species of elm (*Ulmus* spp.). Silvae Genet. 40, 237–243.

Morgenroth, J., Östberg, J., 2017. Measuring and monitoring urban trees and urban forests, in: Ferrini, F., Bosch, C.C.K. van den, Fini, A. (Eds.), Routledge Handbook of Urban Forestry. Routledge, pp. 33–48.

Motiejūnaitė, J., Kutorga, E., Kasparavičius, J., Lygis, V., Norkutė, G., 2016. New records from Lithuania of fungi alien to Europe. Mycotaxon 131, 49–60. https://doi.org/10.5248/131.49

Myers, D.F., Strobel, G.A., 1983. *Pseudomonas syringae* as a microbial antagonist of *Ceratocystis ulmi* in the apoplast of American elm. Trans. Br. Mycol. Soc. 80, 389–394. https://doi.org/10.1016/s0007-1536(83)80034-5

Ogris, N., 2018. Ash dieback and Dutch elm disease: Current situation and prospects in Slovenia. Balt. For. 24, 181–184.

Paal, J., 1998. Rare and threatened plant communities of Estonia. Biodivers. Conserv. 7, 1027–1049. https://doi.org/10.1023/A:1008857014648

Pajares, J., 2004. Elm breeding for resistance against bark beetle. Investig. Agrar. Sist. y Recur. For. 13, 207–216. https://doi.org/10.5424/825

Pajares, J., García, S., Martín, D., Diez, J.J., García-Vallejo, M.C., 2004. Feeding responses by *Scolytus scolytus* to twig bark extracts from elms. Investig. Agrar. Sist. y Recur. For. 13, 217–226. https://doi.org/10.5424/826

Pecori, F., Ghelardini, L., Luchi, N., Pepori, A.L., Santini, A., 2017. Lights and Shadows of a Possible Strategy to Cope with Alien and Destructive Forest Pathogens: the Example of Breeding for Resistance to Dutch Elm Disease in Italy. Balt. For. 23, 255–263.

Pepori, A.L., Bettini, P.P., Comparini, C., Sarrocco, S., Bonini, A., Frascella, A., Ghelardini, L., Scala, A., Vannacci, G., Santini, A., 2018. Geosmithia-Ophiostoma: a New Fungus-Fungus Association. Microb. Ecol. 75, 632–646. https://doi.org/10.1007/s00248-017-1062-3

Phillips, D.H., Burdekin, D.A., 1992. Diseases of Forest and Ornamental Trees. Palgrave Macmillan UK, London. https://doi.org/10.1007/978-1-349-06177-8

Pintson, M.-L., 2015. Ülinakkav ja kiirelt tappev jalakasurm jõudis Tartusse - Uudised - Tänane leht. Postimees.

Pipe, N.D., Brasier, C.M., Buck, K.W., 2000. Evolutionary Relationships of the Dutch Elm Disease Fungus Ophiostoma novo-ulmi to other Ophiostoma Species Investigated by Restriction Fragment Length Polymorphism Analysis of the rDNA Region. J. Phytopathol. 148, 533–539. https://doi.org/10.1046/j.1439-0434.2000.00556.x

Pipe, N.D., Buck, K.W., Brasier, C.M., 1997. Comparison of the cerato-ulmin (*cu*) gene sequences of the Himalayan Dutch elm disease fungus *Ophiostoma himal-ulmi* with those of *O. ulmi* and *O. novo-ulmi* suggests that the *cu* gene of *O. novo-ulmi* is unlikely to ha. Mycol. Res. 101, 415–421. https://doi.org/10.1017/S0953756296002754

Postma, J., Goossen-van de Geijn, H., Schraven, R., 2014. Biological Control of Dutch Elm Disease 2014 (EN) - DutchTrig, in: IOBC "Biocontrol of Plant Diseases". SLU, Uppsala, Sweden.

Rabaglia, R.J., Lanier, G.N., 1983. Effects of multilure components on twig-crotch feeding by European elm bark beetles. J. Chem. Ecol. 9, 1513–1523. https://doi.org/10.1007/BF00988417

Raudsaar, M., Sims, A., Timmusk, T., Pärt, E., Nikopensius, M., 2018. Puuliikide tagavara metsamaal, Aastaraamat Mets 2017. Keskkonnaagentuur. 292 lk.

Relve, H., 2011. Eesti jämedaimad jalakad ja künnapuud. Eesti Loodus, 12.

Rist, E., 2015. Tihemetsa pargi inventuur. Tihemetsa. 25 lk.

Roy, B.A., Alexander, H.M., Davidson, J., Campbell, F.T., Burdon, J.J., Sniezko, R., Brasier, C.M., 2014. Increasing forest loss worldwide from invasive pests requires new trade regulations. Front. Ecol. Environ. https://doi.org/10.1890/130240

Rytkönen, A., Lilja, A., Drenkhan, R., Gaitnieks, T., Hantula, J., 2011. First record of *Chalara fraxinea* in Finland and genetic variation among isolates sampled from Åland, mainland Finland, Estonia and Latvia. For. Pathol. 41, 169–174. https://doi.org/10.1111/j.1439-0329.2010.00647.x

Rytkönen, A., Lilja, A., Petäistö, R.L., Hantula, J., 2008. Irrigation water and *Phytophthora cactorum* in a forest nursery. Scand. J. For. Res. 23, 404–411. https://doi.org/10.1080/02827580802419034

Santini, A., Faccoli, M., 2013. Dutch elm disease and elm bark beetles: a century of association. http://iforest.sisef.org/ 8, 126. https://doi.org/10.3832/IFOR1231-008

Scheffer, R.J., 1990. Mechanisms Involved in Biological Control of Dutch Elm Disease. J. Phytopathol. 130, 265–276. https://doi.org/10.1111/j.1439-0434.1990.tb01177.x

Scheffer, R.J., 1983. Biological control of Dutch elm disease by *Pseudomonas* species. Ann. Appl. Biol. 103, 21–30. https://doi.org/10.1111/j.1744-7348.1983.tb02736.x

Scheffer, R.J., Brakenhoff, A.C., Kerkenaar, A., Elgersma, D.M., 1988. Control of Dutch elm disease by the sterol biosynthesis inhibitors fenpropimorph and fenpropidin. Netherlands J. Plant Pathol. 94, 161–173. https://doi.org/10.1007/BF01978005

Schmidt, O., 2006. Wood and Tree Fungi. Biology, Damage, Protection and Use. Springer-Verlag, Berlin, Heidelberg.

Sell, I., 2019. Roosa võrkheiniku (*Rhodotus palmatus*) kaitse tegevuskava. 27 lk.

Sjöman, H., Östberg, J., Bühler, O., 2012. Diversity and distribution of the urban tree population in ten major Nordic cities. Urban For. Urban Green. 11, 31–39. https://doi.org/10.1016/j.ufug.2011.09.004

Stančin, P., 2018. Karakterizacija gljive *Ophiostoma novo-ulmi* izolirane iz uzoraka nizinskog brijesta s područja Hrvatske (Characterisation of fungus *Ophiostoma novo-ulmi* isolated from field elm samples in Croatia). Sveučilište Josipa Jurja Strossmayera u Osijeku. [In Croatian].

Stennes, M.A., 2000. Dutch Elm Disease Chemotherapy with Arbotect 20-S® and Alamo®, in: Dunn, C.P. (Toim.), The Elms. Springer, Boston, MA, pp. 173–188. https://doi.org/10.1007/978-1-4615-4507-1_10

Stipes, R.J., 2000. The Management of Dutch Elm Disease, in: Dunn, C.P. (Toim.), The Elms - Breeding, Conservation and Disease Management. Kluwer Academic Publishers, Boston, USA, pp. 157–172. https://doi.org/10.1007/978-1-4615-4507-1_9

Stipes, R.J., Campana, R.J., 1981. Compendium of elm diseases, American P. ed, Compendium of elm diseases. American Phytopathological Society., St. Paul, Minnesota.

Süda, I., 2006. Jalaka-maltsaürask *(Scolytus triarmatus* (Eggers, 1912)) – uus üraskiliik Baltikumis. Metsanduslikud Uurim. 44, 112–117.

Sutherland, M.L., Mittempergher, L., Brasier, C.M., 1995. Control of Dutch elm disease by induced host resistance. For. Pathol. 25, 307–315. https://doi.org/10.1111/j.1439-0329.1995.tb01346.x

Swinton, J., Gilligan, C.A., 1999. Selecting hyperparasites for biocontrol of Dutch elm disease. Proc. R. Soc. B Biol. Sci. 266, 437–445. https://doi.org/10.1098/rspb.1999.0657

Synergy Semiochemicals Corporation, 2019. Bark Beetles [WWW dokument]. URL https://semiochemical.com/bark-beetles/ (vaadatud 23.11.19).

Tamm, H., 2007. Taimestik ja taimkate. Eesti Parkide Almanahh I. Muinsuskaitseamet,

Keskkonnaministeerium, lk. 92–97.

Thor, G., Johansson, P., Jönsson, M.T., 2010. Lichen diversity and red-listed lichen species relationships with tree species and diameter in wooded meadows. Biodivers. Conserv. 19, 2307–2328. https://doi.org/10.1007/s10531-010-9843-8

Townsend, A.M., Douglass, L.W., 2004. Evalutation of elm clones for tolerance to Dutch elm disease. J. Arboric. 30, 179–184.

Venturas, M., Fuentes-Utrilla, P., López, R., Perea, R., Fernández, V., Gascó, A., Guzmán, P., Li, M., Rodríguez-Calcerrada, J., Miranda, E., Domínguez, J., González-Gordaliza, G., Zafra, E., Fajardo-Alcántara, M., Martín, J.A., Ennos, R., Nanos, N., Lucena, J.J., Iglesias, S., Collada, C., Gil, L., 2014. Ulmus laevis in the iberian peninsula: A review of its ecology and conservation. IForest 8, 135–142. https://doi.org/10.3832/ifor1201-008

Voeten, J., Postma, J., Cornelissen, B., Houterman, P., Geijn, H.G. de, Goossen-van de Geijn, H., 2009. Biological control of Dutch elm disease. Gewasbescherming (Plant Prot. 40.

Voolma, K., Mandelshtam, M.Y., Shcherbakov, A.N., Yakovlev, E.B., Õunap, H., Süda, I., Popovichev, B.G., Sharapa, T. V., Galasjeva, T. V., Khairetdinov, R.R., Lipatkin, V.A., Mozolevskaya, E.G., 2004. Distribution and spread of bark beetles (Coleoptera: Scolytidae) around the Gulf of Finland: a comparative study with notes on rare species of Estonia, Finland and North-Western Russia. Entomol. Fenn. 15, 198–210.

Voolma, K., Õunap, H., Süda, I., 2000. Eesti putukate levikuatlas, 2: Ürasklased – Scolytidae. Eesti Loodusfoto, Tartu. 84 lk.

Webber, J.F., 2004. Experimental studies on factors influencing the transmission of Dutch elm disease. Invest Agrar Sist Recur 13, 197–205.

Webber, J.F., 1987. Influence of the d2 factor on survival and infection by the Dutch elm disease pathogen *Ophiostoma ulmi*. Plant Pathol. 36, 531–538. https://doi.org/10.1111/j.1365-3059.1987.tb02270.x

Zalapa, J.E., Brunet, J., Guries, R.P., 2008. Genetic diversity and relationships among Dutch elm disease tolerant *Ulmus pumila* L. accessions from China. Genome 51, 492–500. https://doi.org/10.1139/G08-034

CURRICULUM VITAE

First name:	Liina
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Address:	Institute of Forestry and Rural Engineering, Estonian University of Life Sciences, Kreutzwaldi 5, 51014 Tartu
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Education:

2014–2021	Estonian University of Life Sciences, Institute of Forestry and Rural Engineering, PhD student
2009–2011	Luua Forestry School, arboriculture
2004–2007	Luua Forestry School, landscaping
2002–2004	Räpina Gardening School, landscape design
1984–1987	Leningrad Institute of agriculture, agronomist in horticulture
1981–1984	Estonian Academy of Agriculture (nowadays Estonian University of Life Sciences)
1970–1981	Miina Härma nim. Tartu 2. Keskkool

Academic degree:

1987	higher education (MSc), trained horticultural agronomist

Professional employment:

01.05.2019–...	Estonian University of Life Sciences, chief specialist
05.10.2016–30.04.2019	Estonian University of Life Sciences, Institute of Agricultural and Environmental Sciences, Courses: Greenspace management and Woody Plants in urban greening
2012–2016	Pärnu County Vocational Education Center, teacher (0,30)
2010–2015	Olustvere School of Service and Rural Economics, teacher (0,50)
2007–2011	Räpina Gardening School, teacher (1,00)

Research interests: forest pathology, arboriculture, horticulture

Foreign languages: English, Russian

Training and special courses:

2014	Course on using the PlutoF cloud, Biodiversity Informatics Working Group, Pärnu, Dec. 8-10
2015	Monitoring tree reaction to environment: tools and their application in forest, BOVA course in University of Agriculture, Latvia, June 15 to 19
2017	Erasmus+ Participation in the UEF staff training week in Eastern University of Finland, June 05.-09. lst international plener "Green art - Topiary", Klaipeda, Lithuania, June 13.-15. Forestry doctoral school June 19.-22. Salaspils, Latvia
2018	Visiting Starhill Forest Arboretum, Illinois, USA October 25.-27.
2019	Erasmus+ Participation in the English course "CLIL for Educators", Galway, Ireland, August 19.-23.
2020	Forest disturbances under climate change. online seminar by University of Helsinki, December 2., 8. and 14.
2021	Supportive teaching in higher education: supervising student work, University of Tallinna Ülikool, April 01. Trees & Society. Online Conference organised by Arborcultural Association , UK. September 06.-07.

Projects:

2018-	PSG136 "Massive invasions of forest pathogens to Northern Europe: early detection of new pathogens, determination of the pathways and modes of their arrival and search of the possibilities of their obstruction (1.01.2018−31.12.2021)", Rein Drenkhan, Estonian University of Life Sciences.
2020	L200047PKLA Development contract No 20-2554) "Dendropark inventory (1.07.2020−1.10.2020)", Liina Jürisoo, Estonian University of Life Sciences, Institute of Agricultural and Environmental Sciences, Chair of Landscape Architecture.
2018-2020	T180210MIME (15599) Prevalence and danger of DED in Estonian forests and green areas (Jalakasurma levik ning ohtlikkus Eesti metsades ja haljasaladel

(1.12.2018−1.12.2020), Rein Drenkhan, Estonian University of Life Sciences, Institute of Forestry and Rural Engineering.

2019 V190091MIME (Agreement No 2019/7) "Elm disease in Germany and Estonia: Investigation of resistant elms and microorganisms as biocontrol (1.03.2019−31.10.2019)", Tiia Drenkhan, Estonian University of Life Sciences, Institute of Forestry and Rural Engineering.

2019 L190137MIME Monitoring the number of bark beetles (Scolytus sp.) and reducing their spread in Tallinn (Maltsaüraskite (Scolytus sp.) arvukuse monitooring ja nende leviku vähendamine Tallinnas (13.11.2019−1.12.2019)), Liina Jürisoo, Estonian University of Life Sciences, Institute of Forestry and Rural Engineering, Chair of Silviculture and Forest Ecology.

2018 ASTRA project Value Chain Bioeconomy, Doctoral School of Earth Sciences and Ecology "Fieldwork in Eastern Europe, Belarus-Ukraine. 10.-18.06.2018

2018 Research of Järvselja Study and Experimental Forest Foundation. Establishment of experimental area of elms in Järvselja Study and Experimental Forest Foundation, põhitäitja, principal

2012-2014 8-2/T12051PKMA (ELRI-177) "Tartu, Rezekne, Pskov: Green Management for Urban Development & Planning in EE-LV-RU Border Capitals (1.05.2012−31.10.2014)", Jekaterina Balicka, Estonian University of Life Sciences, Institute of Agricultural and Environmental Sciences, Department of Landscape Architecture.

MSc dissertations supervised:

2018 Triin Kask, Puud Eesti linnaruumis: kasutamine ja hooldus (Trees in Estonian urban environment: use and care), Estonian University of Life Sciences.

2018 Elvi Liiv, Linnade avatud alade hoolduse muutmine ökoloogilisuse tõstmise eesmärgil Eesti Maaülikooli linnaku näitel (Increasing ecological values of city open spaces by changing maintenance intensity in Estonian University of Life Sciences campus), Estonian University of Life Sciences.

ELULOOKIRJELDUS

Eesnimi: Liina
Perekonnanimi: Jürisoo
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Aadress: Metsandus- ja maaehitusinstituut,
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Hariduskäik:

2014–2021	Eesti Maaülikool, metsandus- ja maaehitusinstituut, metsandus, doktoriõpe
2009–2011	Luua Metsanduskool, arborist
2004–2007	Luua Metsanduskool, maastikuehitus
2002–2004	Räpina Aianduskool, maastikukujundus
1984–1987	Leningradi Põllumajanduse Instituut, aianduse teaduskond, 3.-5. kursus õpetatud aiandusagronoom
1981–1984	Eesti Põllumajanduse Akadeemia, agronoomia-teaduskond, 1.-3. kursus
1970–1981	Miina Härma nim. Tartu 2. Keskkool

Teaduskraad:

1987	õpetatud aiandusagronoom

Teenistuskäik:

2019-2020	Eesti Maaülikool, peaspetsialist
2016-2019	Eesti Maaülikool, nooremteadur
2012–2016	Pärnumaa Kutsehariduskeskus, arboristide kutseõpetaja
2010–2015	Olustvere Teenindus- ja Maamajanduskool, aianduse kutseõpetaja
2007–2011	Räpina Aianduskool, kutseõpetaja

Teadustöö põhisuunad:
Puittaimede haigused ja -kaitse, arboristika, aiandus

Võõrkeelte oskus: inglise, vene

Täiendkoolitused:

2014	Loo, toimeta ja publitseeri eu- ja prokarüootide andmebaase veebi põhiselt, PlutoF pilve kasutamine, Elurikkuse informaatika töörühm, Pärnu, 8-10 detsember
2015	Puu reaktsioon keskkonnale: tööriistad ja newnde kasutamien metsas, BOVA kursus Lätis Põllumajanduse Ülikoolis, 15.-19.06
2017	Erasmus+ osalemine ülikoolide töötajate õppenädalal Ida-Soome Ülikoolis, 05.-09.juunil Osalemine pleenumil „ Topiaar kui roheline kunst“, Klaipeda, Leedu, 13.-15. juuni
2019	Erasmus+ osalemine inglise keele kursusel “CLIL pedagoogidele”, Galway, Iirimaa, 19.-23.08
2020	Metsahäiringud kliimamuutuste mõjul. Online seminar, Helsinki ülikool, 2., 8. ja 14. detsember
2021	Õpetamise toetamine kõrgkoolis: üliõpilastööde juhendamine, Tallinna Ülikool 01.04.2021 Puud ja ühiskond. Online konverents, Inglise Arboristide Assotsiatsioon, 06-07.09.2021

Projektid:

2018-	PUT136 “Metsapatogeenide mass-invasioonid Põhja-Euroopasse: uute patogeenide varane tuvastamine, nende saabumisteede ja -viiside määratlemine ning tõkestusvõimaluste otsimine”, täitja
2020	L200047PKLA (Arendustöö leping nr 20-2554) “Dendropargi inventeerimine (1.07.2020−1.10.2020)”, põhitäitja.
2018-2020	T180210MIME (15599) “Jalakasurma levik ning ohtlikkus Eesti metsades ja haljasaladel, põhitäitja.
2019	V190091MIME (Agreement No 2019/7) “Jalakasurm Saksamaal ja Eestis: resistentsete jalakate ja mikroorganismide kui biotõrje uurimine” (Elm disease in Germany and Estonia: Investigation of resistant elms and microorganisms as biocontrol (1.03.2019−31.10.2019), täitja

2019	L190137MIME "Maltsaüraskite (*Scolytus* sp.) arvukuse monitooring ja nende leviku vähendamine Tallinnas", põhitäitja
2018	ASTRA projekt Väärtusahelapõhine biomajandus, Maateaduste ja ökoloogia doktorikool. Välitööd Ida-Euroopas, Valgevenes ja Ukrainas. Toimumise kuupäevad: 10.-18.06.2018
2018	SA Järvselja ÕKMK teadusuuringud. Perekond Jalaka katseala rajamine SA Järvselja Õppe- ja Katsemetskonda, põhitäitja
2016	EFI SSV stipendium "Jalakasurma levik Eesti idanaabruses (Spread of the Dutch Elm Disease in the Eastern Neighbourhood of Estonia)". Dates of the visits: June 10–14, July 5–7 and July 21–23, 2016
2012-2014	8-2/T12051PKMA (ELRI-177) "Tartu, Rezekne, Pihkva: Linnade arendamise ja planeerimise roheline juhtimine EE-LV-RU piiriäärsetes pealinnades (1.05.2012−31.10.2014)", täitja

Juhendatud magistritööd:

2018	Triin Kask, Puud Eesti linnaruumis: kasutamine ja hooldus.
2018	Elvi Liiv, Linnade avatud alade hoolduse muutmine ökoloogilisuse tõstmise eesmärgil Eesti Maaülikooli linnaku näitel (kaasjuhendaja: Gloria Niin)

LIST OF PUBLICATIONS

Clarivate Web of Science database

Mullett, M. S.; Adamson, K.; Bragança, H; Bulgakov, T. S.; Georgieva, M.; Henriques, J.; **Jürisoo, L.**; Laas, M.; Drenkhan, R. (2018). New country and regional records of the pine needle blight pathogens *Lecanosticta acicola*, *Dothistroma septosporum* and *Dothistroma pini*. Forest Pathology, 48 (e12440).10.1111/efp.12440.

Jürisoo L., Adamson K., Padari A., Drenkhan R. 2019. Health of elms and Dutch elm disease in Estonia. Eur J Plant Pathol. 154:823–841. doi.org/10.1007/s10658-019-01707-0

Jürisoo, L.; Süda, I.; Agan, A.; Drenkhan, R. 2021. Vectors of Dutch Elm Disease in Northern Europe. Insects, 12, 393. https://doi.org/10.3390/insects12050393

Jürisoo, L., Selikhovkin, A.V., Padari, A., Shevchenko, S.V., Shcherbakova, L.N., Popovichev, B.G., Drenkhan, R. 2021. The extensive damages of elms by Dutch elm disease agents and their hybrids in north-western Russia. Urban Forestry and Urban Greening, 63, 127214. DOI: 10.1016/j.ufug.2021.127214

Jürisoo, L., Padari, A. Drenkhan, R. 2021. Jalakasurma levikust ja ohtlikkusest Eestis (Spread and riskiness of Dutch elm disease in Estonia). Forestry Studies/Metsanduslikud Uurimused. 74. DOI: 10.2478/fsmu-2021-0006 [In Estonian]

Chapter in a peer-reviewed book

Drenkhan, R., Agan, A., Palm, K., Rosenvald, R., **Jürisoo, L.**, Maaten, T., Padari, A., Drenkhan, T. 2017. Overview of ash and ash dieback in Estonia. In: Vasaitis, R., Enderle, R. (Ed.). Dieback of European 122 Ash (Fraxinus spp.) – Consequences and Guidelines for Sustainable Management. Sweden, Uppsala, 115–124.

Popular-scientific publications

Jürisoo, L. 2012. Nudi- ja vormipuud: kujundamine ja majandamine (2012). Luua Metsanduskooli Artiklid ja uurimused XI, lk 5-10, leitav http://luua.kovtp.ee/documents/105873/1751433/artiklid2012.pdf/4858c995-c906-43c0-af8d-255760063cb2

Jürisoo, L. jt 2020. PUITTAIMED HALJASTUSES. Osa 1: Terminid ja määratlused. EESTI STANDARD. Eesti Standardikeskus, 18 lk

Jürisoo, L. jt 2020. PUITTAIMED HALJASTUSES. Osa 2: Ilupuude ja -põõsaste istikute kvaliteedinõuded. EESTI STANDARD. Eesti Standardikeskus, 26 lk

Jürisoo, L. jt 2020. PUITTAIMED HALJASTUSES. Osa 3: Ehitusaegne puude kaitse. EESTI STANDARD. Eesti Standardikeskus, 39 lk

Jürisoo, L. jt 2020. PUITTAIMED HALJASTUSES. Osa 4: Puu-hooldustööd. EESTI STANDARD. Eesti Standardikeskus, 44 lk

Jürisoo, L., Drenkhan, R. 2021. Jalakasurm kahjustab Eesti jalakaid rohkem kui kunagi varem. Eesti Loodus 72(9), 36-38

PRESENTATIONS

Presentations at international conferences and meetings

Jürisoo, L. 2015. Bridges across the Baltic: International Cooperation on Arboricultural Standards. ISA annual conference "Where the Trees Meet the Seas*",* August 11, 2015, Florida, USA.

Jürisoo, L. 2016. Current Tree Health Issues and Structural Pruning. Annas Tree School seminar „Koks Meža" (tree forest), February 19, 2016, Latvia.

Jürisoo, L., Drenkhan R. 2018. Dutch elm disease in Estonia. Forestry conference for Baltic PhD students, April 26-27, 2018, Sokka, Estonia.

Jürisoo, L., Drenkhan R. 2018. Dutch elm disease in Estonia. LIFE+ ELMIAS Ash and Elm, and IUFRO WP 7.02.01 Root and Stem Rots Conference (LIFE-IUFRO), August 26. - September 1., 2018, Uppsala and Gotland, Sweden.

Jürisoo, L. 2019. Elms and Dutch elm disease in several East European countries. Seminar in University of Göttingen. April 09, 2019, Göttingen, Germany.

Jürisoo, L., Drenkhan R. 2019. Elms and Dutch elm disease in north-eastern Europe part of Europe, IUFRO WP 7.02.02 & 7.02.03 PHYLLOSPHERE DISEASES, May 10, 2019, Figline Valdarno, Italy.

Jürisoo, L., Drenkhan R. 2019. The situation of elms and Dutch elm disease in Estonia. Seminar of pathology in LUKE, November 14, 2019, Helsinki, Finland.

Jürisoo, L., Drenkhan R. 2019. Health of elms and Dutch elm disease in Estonia and Leningrad region. Seminar in SPY (Finnish arborist union), November 29, 2019, Tampere, Finland.

Presentations at local meetings

Jürisoo, L., Drenkhan R. 2019. Jalaka-siugsuu**,** tekitajad, võimalik levik Eestis ja lähiriikides. Aleksei Paiveli 90. sünniaastapäevale pühendatud konverents Tallinna Botaanikaaias, 18. september, 2019.

VIIS VIIMAST KAITSMIST

KRISTIINA AUN
RAIETE LÜHIAJALINE MÕJU SÜSINIKU VOOGUDELE JA VARUDELE ERINEVATES EESTI METSAÖKOSÜSTEEMIDES
SHORT-TERM EFFECT OF FELLING ON CARBON FLUXES AND STORAGES IN DIFFERENT ESTONIAN FOREST ECOSYSTEMS
Professor Veiko Uri
27. august 2021

PRIIT KARIS
TOITUMUSE, LIPOMOBILISATSIOONI JA INSULIINIRESISTENTSUSE SEOSED PIIMALEHMADEL
RELATIONSHIPS BETWEEN BODY CONDITION, LIPOMOBILIZATION AND INSULIIN RESISTANCE IN DAIRY COWS
Doktor Hanno Jaakson, doktor Katri Ling, doktor Meelis Ots
31. august 2021

HARES KHAN
KALTSIIDI AVAVEELINE SADENEMINE: PÕHJUSED JA TAGAJÄRJED GLOBAALSES JA KOHALIKUS VAATES
PELAGIC CALCITE PRECIPITATION IN LAKES: FROM A GLOBAL TO A LOCAL PERSPECTIVE ON ITS DRIVERS AND IMPLICATIONS
Doktor Biel Obrador (University of Barcelona, Hispaania), doktor Alo Laas
16. september 2021

HEIKI LILL
ERINEVATE ENERGIA SALVESTUSTEHNOLOOGIATE UUDSED RAKENDUSPÕHIMÕTTED LIGINULLENERGIAHOONETES
NOVEL APPLICATION PRINCIPLES FOR ENERGY STORAGE TECHNOLOGIES IN NEARLY ZERO ENERGY BUILDINGS
Teadur Alo Allik, professor Andres Annuk
29. oktoober 2021

MARGUS ARAK
AMMENDATUD FREESTURBAVÄLJADEL KASVATATAVA AHTALEHISE MUSTIKA MASINVILJELUSTEHNOLOOGIA
CULTIVATION TECHNOLOGY FOR LOW-BUSH BLUEBERRY CULTIVATION IN MILLED PEAT FIELD PLANTATIONS
Professor Jüri Olt
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